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Chapter 4

In vitro propagation of Eucalyptus species

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Contents

KEY WORDS: *Eucalyptus,* eucalypt, micropropagation, clonal field trials, *Phytophthora cinnamomi,* root architecture, *in vitro* rooting, salt tolerance, transformation, protoplasts.

LIST OF ABBREVIATIONS:

AFOCEL	Association Foret Cellulose
BAP	Benzylamino-purine
BT	*Bacillus thuringensis*
CSIRO	Commonwealth Scientific and Industrial Research Organisation
GA_3	Gibberellin A_3
IAA	Indole acetic acid
IBA	Indole butyric acid
MS	Murashige and Skoog (1962) medium
NAA	Naphthalene acetic acid
RR	Resistant jarrah seedlings from families on average, resistant to dieback
RS	Resistant jarrah seedlings from families on average, susceptible to dieback
SS	Susceptible jarrah seedlings from families on average, susceptible to dieback

LIST OF SPECIES (see end of chapter).

Introduction

The importance of eucalypts and reasons for tissue culture

Eucalypts are Australia's most distinctive plant group. They are contained within the genus *Eucalyptus* which consists of over 500 named species, with more as yet unnamed (Brooker & Kleinig 1983; 1990 Chippendale 1988). The natural distribution of the genus is almost completely confined to the Australian continent and Tasmania with only two species, *E. deglupta* and *E. urophylla*, occuring naturally in other countries. Since European settlement of Australia, seeds of eucalypts have been sent to countries throughout the world and they are now commonly grown in tropical and temperate areas for timber, pulp wood, eucalyptus oil, fuelwood, charcoal and as ornamentals.

Exploitation of eucalypts outside Australia was initiated by the French. During the nineteenth century, eucalypts were planted in Europe and North America, and European imperial governments introduced them to colonies in South America, Africa and Asia. The presence of eucalypts in some of these countries is now so familiar to the native peoples that many consider them to be indigenous (Zacharin 1978).

Although eucalypts in early plantations often grew very quickly the wood was sometimes of poor quality due to wood splitting and distortion (Clarke 1957; Penfold & Willis 1961; Pryor 1976). In many cases this was because the species chosen were inappropriate for local climatic and edaphic conditions (Evans 1980; Durand-Cresswell *et al.* 1982), the trees had been planted for the wrong purposes (Penfold & Willis 1961; Pryor 1976), or given incorrect fertilisers (Savory 1962; Stone 1968).

The poor quality of the wood led to a slump in enthusiasm for growing eucalypts until about 1945 when world demand for pulpwood started to increase (Pryor 1976). Today the major uses of eucalypt wood are for fuelwood and pulpwood. There has been a 150 fold increase in pulpwood production from eucalypts since the early 1960s (Molleda 1984). They are now the most widely planted hardwood group in the world (Boland *et al.* 1984; Eldridge *et al.* 1993).

Eucalypt plantations outside Australia

Eldridge *et al.* (1993) estimate that there are over 6,000,000 hectares of eucalypt plantations world wide. The figures they give, updated for Chile by Jayawickrana *et al.* (1993), Morocco (M. Idrissi pers. comm. 1992) and for China by Houran *et al.* (1994) show that there are large areas planted in South America where countries such as Brazil have over 3 million hectares, Argentina over 250,000 hectares, Chile over 170,000 hectares and Peru 90,000 hectares. On the African continent countries such as Angola (390,000), Ethiopia (250,000), Morocco (200,000), the Congo and the Republic of South Africa (470,000) have substantial areas dedicated to eucalypts. Other countries such as China, Portugal, India, Spain and Uruguay have areas under plantations of between 100,000 and 600,000 hectares.

Despite the large areas that are planted, the number of species utilised in these plantations is quite restricted. The 4 major species are *E. globulus*, *E. grandis*, *E. camaldulensis* and *E. tereticornis* or hybrids developed from these species.

Important hybrids include the Mysore gum derived from *E. tereticornis* in India. The true origin of this hybrid is unclear (Eldridge *et al.* 1993) but it covers over 500,000 hectares in plantations. Examples of other important hybrids include *E. grandis* x *urophylla*, *E. grandis* x *tereticornis*. Attempts are now in progress to develop further eucalypt hybrids to improve production through combining characteristics of different species such as productivity, wood quality, frost tolerance etc. (Eldridge *et al.* 1993; Stephens *et al.* 1993). Concerted efforts are being made to produce more frost tolerant plants by crossing frost tolerant species such as *E. gunnii* and *E. nitens* with species such as *E. globulus* which have good pulping quality (De Little *et al.* 1992).

Australian plantations

The utilisation of eucalypts in Australia has relied heavily upon exploitation of naturally occurring stands. Plantations have not been prominent in terms of productivity or acreage but this is changing rapidly. Over the 1984-1994 period the number of hectares planted to eucalypts is estimated to have grown from 39,107 (Yearbook 1984) to 101.663 (Stephens *et al.* 1993; Yearbook 1994). This increase is required to relieve pressure on the exploitation of native forests and to supply a more consistent, quality product (Kerruish & Rawlins 1991).

Eucalypt breeding programs

Eucalypt breeding programs have concentrated on selection of appropriate provenances for particular growing conditions, selecting and propagating high yielding individuals, improving wood quality, salt tolerance, frost tolerance and oil yield. In many of these programs vegetative propagation through cuttings or grafting has played an important role; in fewer, tissue culture has also been important. For tissue culture to become more prominent there is a clear need to improve tissue culture protocols.

Use of tissue culture

Past tissue culture research with eucalypts has mainly concentrated on developing techniques for clonal propagation with the aim of using these *in vitro* clonal plants instead of, or alongside those from conventional vegetative propagation. The current use of tissue culture of eucalypts can be placed into three broad areas:

1 Clonal propagation of elite individuals for direct planting, the establishment of clonal seed orchards or of clonal hedges for cuttings.

2 Manipulation of breeding lines for further selection or for cross pollination.

3 Bioengineering including hybridisation through protoplast fusion and the production of genetically engineered plants.

Micropropagation and tissue culture of eucalypts has been extensively reviewed by Le Roux and van Staden (1991) and Lakshmi Sita (1993) and the methods recently summarised by McComb (1995). Consequently this review concentrates on the application of tissue culture in eucalypts giving examples of use of tissue cultured eucalypts in the field, and providing detailed information only on those species that are most widely used in plantations.

History of eucalypt tissue culture

Although the earliest publications on eucalypt tissue culture came from France (Jaquiot 1964a, b), America (Sussex 1965) and India (Aneja & Atal 1969), there is no doubt that the Father' of *Eucalyptus* tissue culture is Dr Ron de Fossard. From the early 1970s he and his co-workers from the University of New England produced a valuable series of papers on callus culture, organogenesis, media for shoot culture and techniques for rooting (see references under de Fossard 1974 *et seq*; Lee & de Fossard 1974; Cresswell & de Fossard 1974; Barker *et al.* 1977; Gorst *et al.* 1983). He elucidated the value of using seedling explants, the problems in use of the mature ones, and need for rejuvenation. In his talks and writings he laid the basis for future research, emphasised the need for systematic, rather than *ad hoc* evaluation of media ingredients (de Fossard *et al. 19*74a), and described ingenious methods for low-cost tissue culture (de Fossard 1978).

In vitro propagation of eucalypts was also achieved in the 1970s in France, and by French foresters working in North America (Franclet & Boulay 1982). They built on their previous expertise in rejuvenation of eucalypts for provision of suitable shoots for cuttings (Franclet 1956). Research is continuing today by AFOCEL, in Universities in France and regions of French influence in north and central Africa.

By the 1980s it was clear that micropropagation had potential for mass propagation of elite eucalypts (Gupta *et al.* 1981; Lakshmi Sita 1982; Bennett & McComb 1982). In Australia application of micropropagation began with selection (Thomson 1981; 1988) and tissue culture of salt tolerant lines of *E. camaldulensis* (Hartney 1982) and other species (McComb & Bennett 1986). Research was also initiated to select and propagate jarrah (*E. marginata*) resistant to *Phytophthora cinnamomi* as described in more detail below.

At present Australian research on tissue culture of eucalypts is concentrated in the Co-operative Research Centre for Temperate Forestry in Hobart, Murdoch University, Edith Cowan University and Alcoa (Aust) in Western Australia. Research on specific topics being done elsewhere includes mineral nutrition *in vitro* (University of New England), rare and endangered species (Kings Park and Botanic Gardens, Perth; and Black Hill Flora Centre, Adelaide), horticultural species (Knoxfield, Victoria), genetic engineering (CSIRO Division of Plant Industry) use of *Agrobacterium rhizogenes* for rooting (Florigene, Melbourne) and automation (ForBio Research Queensland).

The widespread use of eucalypts in plantations has generated research on eucalypt tissue culture on every continent – North and South America, North and South Africa, China and India as well as in Europe and Australia. Countries with few or no eucalypts such as Japan have also contributed to eucalypt tissue culture research because of the value of their investments in overseas plantations or interest in secondary metabolite production. In India interest has centred not only on shoot cultures but also on suspension cultures and embryogenesis. Research on species of interest to India has recently been reviewed by Lakshmi Sita (1993).

Recent years have seen the closure or severe curtailment of eucalypt tissue culture research programmes at for example Shell (UK), Florigene (Melbourne) and Celbi (Portugal). This is due in part to the world wide economic recession, but also in part to the failure to develop for the major plantation species, methods for micropropagation that are cost effective compared with use of cuttings. Present work on eucalypts *in vitro* is frequently slanted towards automation or genetic engineering; areas that are far more costly to research than micropropagation, but which may have great economic benefits in the longer term.

Case studies of the application of tissue culture

Jarrah (*Eucalyptus marginata* Donn ex Sm.)

Jarrah is Western Australia's most important timber species. It is endemic to south-Western Australia where it grows on soils ranging from deep, leached sands of the coastal plain to heavier lateritic soils further inland. The eastern boundary of its distribution lies near the 635 mm isohyet. In the woodlands of the coastal plain it is co-dominant with other eucalypts and *Banksia* species. Further inland it is the dominant species of an open dry sclerophyll forest and often forms almost pure stands.

In the more fertile areas, jarrah can reach a height of up to 40 m. The wood is hard and durable and resistant to termite and fungal attack. In Western Australia, jarrah is used for structural purposes but in other states and overseas it is popular for cabinet making, panelling and ornamental woodwork. Due to its durability it has been used for road foundations in many areas throughout the world and is still used as railway sleepers.

Tissue culture of jarrah

(a) *Callus Induction, Regeneration*

As with other eucalypts callus induction and organogenesis is readily achieved from juvenile explants taken from aseptic seeds. However, when mature explants are used, more variable results are obtained. Callus induction is possible from most mature vegetative explants but successful shoot regeneration is only obtained in callus produced from stamen filaments and styles (Bennett & McComb 1982; Bennett 1988).

Figure 1: Jarrah and marri forest at Dwellingup (90 km SE of Perth). Jarrah trees have been killed by *Phytophthora cinnamomi* and the area logged.

(b) *Shoot Growth*

Initiation of cultures using nodes with naked buds, from trees growing in the forest, is achieved using benzalkonium chloride as a sterilising agent. The best time of the year to obtain aseptic shoots is in spring and summer when the plants have new seasons growth (McComb & Bennett 1986).

When shoots are obtained *in vitro*, from either callus or naked buds, all genotypes grow on a medium solidified with 0.8 % agar containing Murashige and Skoog (1962) (MS) minerals and vitamins, 2.0% sucrose, 2.5 μM benzylamino purine and 1.25 μM naphthalene acetic acid with a pH of 5.8.

(c) *Root Induction*

A root induction medium developed for jarrah contains quarter strength MS macronutrients, half Fe, full strength micronutrients, 2% sucrose and 10 μM indole butyric acid. As with other eucalypts, root induction from mature explants is difficult. However, when shoot cultures from mature trees are maintained in culture for long periods the response to root induction medium improves (McComb & Bennett 1982). This result is similar to that reported for other eucalypts (Gupta *et al.* 1981) as well as other woody species (McCown & Lloyd 1982; Tricoli *et al.* 1985). McCown & McCown (1987) use the term 'stabilisation' to describe this change, which may be associated with rejuvenation. In the case of jarrah, when mature shoots stabilise and form roots *in vitro* they do not necessarily acquire rejuvenation for all characteristics associated with the juvenile condition. For example they show mature rather than juvenile leaves at ground level (Bennett *et al.* 1986).

Successful transfer of the plantlets to soil depends upon a good root system and a healthy shoot. For some clones it is necessary to initiate roots on an auxin medium then transfer them to an auxin free medium to obtain plantlets with both a good root system and a healthy shoot.

(d) *Genotypic Variation*

The performance at all stages of the tissue culture process is genotype dependent. There is no correlation between shoot regeneration, shoot growth and multiplication and/or ease of root induction. While some genotypes are easily taken through the whole tissue culture cycle others are impossible or very difficult. Those considered most valuable are often most difficult to clone!

Problems with *Phytophthora cinnamomi*

(a) *Jarrah Dieback Disease*

In 1965 *P. cinnamomi* was first isolated in association with jarrah dieback disease (Podger 1972). By 1972 approximately 80,000 hectares of forest had been severely damaged and this area was increasing by about 4% each year (Podger 1972). By the early 1980s approximately 14% of the jarrah forest was affected by dieback (see Weste & Marks 1987; Dell and Malajczuk 1989; Shearer & Tippett 1989 for reviews).

From research into the epidemiology, ecology and physiology of the fungus in the jarrah forest it became obvious that the response of individual jarrah plants to infection was variable. Some jarrah trees were observed surviving on dieback sites and others which had been inoculated with *P. cinnamomi* on a number of occasions showed no symptoms of the disease (Rockel 1977; Tippett *et al.* 1985). Studies which measured lesion length development (Grant & Byrt 1984) and responses to pot inoculation (Podger 1972) showed considerable variation between individual plants and provenances.

Ramets micropropagated from trees that had survived on dieback sites for long periods (>20 years) despite being inoculated with *P. cinnamomi,* were inoculated *in vitro* and in pot experiments, and displayed a consistent response to infection. Different clonal lines responded in different ways to infection (Bennett *et al.* 1993). Producing clonal lines from mature trees such as these is difficult (McComb & Bennett 1982).

In 1985 Mr M Stukely at the Western Australian Department of Conservation and Land Management initiated a programme to screen for resistance to *P. cinnamomi* in jarrah seedlings. The techniques used were similar to those developed for *Pinus radiata* for resistance to *P. cinnamomi* (Butcher *et al.* 1984) and utilised both under bark and soil inoculation (McComb *et al.* 1990; Stukely & Crane 1994).

Half-sib families of jarrah seedlings 12 months old were ranked as having high, intermediate or low resistance to *P. cinnamomi* on the basis of the mean lengths of the lesions developed after underbark inoculation with *P. cinnamomi*,

and the percentage of deaths after soil inoculation (Stukely & Crane 1994). Individual seedlings were selected from families on the following basis:

- susceptible individuals from a susceptible family (SS)
- resistant individuals from a susceptible family (RS)
- resistant individuals from a resistant family (RR)

The authors then micropropagated these selections at Murdoch University. Being juvenile, the plants could be cloned more readily than mature trees. Inoculation of clones with *P. cinnamomi* under laboratory conditions has allowed lesion extension in roots and production of secondary products such as lignin and phenolics to be examined (Cahill & McComb 1992; Cahill *et al.* 1991, 1993). The results so far indicate that clones in the RR category are showing a response similar to that seen in a field resistant species, *Eucalyptus calophylla*, those in the RS category show a variable response and those in the SS category show a susceptible response, comparable with that seen in unselected jarrah seedlings (Cahill & McComb 1992; Cahill *et al.* 1993).

Clones have been planted in a number of field trials and inoculated with *P. cinnamomi*. Clonal survival reflects the ranking for susceptibility or resistance determined under glasshouse conditions. After 5 years RR clones have very low mortality with no deaths occurring after the first summer; RS clones have intermediate mortalities and SS clones have high mortalities. *P. cinnamomi* was recovered from roots of most of the dead plants and has been consistently recovered from the soil (McComb *et al.* 1990; 1994).

Insect problems

Jarrah leafminer *Perthida glyphopa* was recognised as a problem in the 1920s (Newman & Clark 1926) and has since caused widespread damage to jarrah in the southern jarrah forest. Individual trees in the forest resist leafminer attack by various mechanisms such as having the ability to inhibit larval development or eject eggs from the leaf (Wallace 1970; Mazanec 1985; 1989). Clones produced from one of these resistant trees display the same level of resistance as the parent tree and are equally able to restrict the growth of the developing larvae (Bennett *et al.* 1992a).

Current research into insect herbivory on jarrah is aimed at establishing the heritability of resistance to insect pests and the role of the environment in determining resistance. This information will indicate whether insect resistance can be bred into jarrah. Some of the clones that have been selected for *P. cinnamomi* resistance have also been included in this work and their resistance to insect attack will be determined.

Figure 2: Bauxite pit rehabilitation with jarrah at Jarrahdale (50 km SE of Perth). Clones are two years old and have been infected with *Phytophthora cinnamomi*. The tall plants are from resistant lines (RR); the small dead plant (left front) is from a susceptible line (SS); the pale plant (right front) is a 'resistant' plant from an otherwise susceptible family (RS) and has since died (photo M. Stukely).

Future prospects for clonal jarrah

The results from initial field trials of dieback resistant jarrah are very encouraging and further experiments are being conducted to increase the number of genotypes that show a high level of resistance in the field, and examine the response of clones in several different environments. Heritability of resistance to *P. cinnamomi* has been found to be high (Stukely & Crane 1994), and specific combining ability will be examined after hand-crossing clones. The flowering time of each resistant clone will also be recorded so that seed orchards with appropriate mixes of clones can be established.

Various aspects of the tissue culture protocol for jarrah must be improved if clones are to be used for direct field planting. There are at present a number of valuable plants that are very difficult or impossible to clone. Developing individual media or methods for particular clones may be necessary but general improvement of the percentage of rooting, and survival after transfer to soil are the highest priorities.

Eucalypts tolerant of saline waterlogging

In 1986 a Tree Technology Project was set up to develop Australia's natural resources in salt tolerant eucalypt genotypes. The research was a collaborative effort between Alcoa (Australia), CSIRO Division of Forestry, The University of

Figure 3: A clonal line of selected salt tolerant *Eucalyptus camaldulensis* on saline land at Boyup Brook (270 kms S of Perth). Trees are five years old (photo D. Bell).

Western Australia, Murdoch University and Plantex (Australia). The CSIRO tree and seed centre collected seeds from eucalypt species growing in saline waterlogged areas, and 6 month old seedlings were screened at the University of Western Australia in glasshouse trials for tolerance to saline, waterlogged conditions of up to 600 mM NaCl (van der Moezel & Bell 1987; van der Moezel *et al.* 1988; 1992;). Previous work (Thomson 1981; 1988) on selection of *E. camaldulensis* tolerant to salt, utilised aerated saline solutions, but it was considered that for use in rehabilitation of secondarily salinised discharge zones, plants would need to be tolerant of both salinity and waterlogging. The genotypes showing superior tolerance at the end of a trial had the salt washed from their pots and were allowed to recover to provide explants suitable for tissue culture. This usually occurred within 4-6 months of the end of the salt waterlogging trial. Mother plants were kept in a glasshouse and regularly treated with fertiliser and fungicide to keep shoots in good condition for tissue culture.

Occasionally shoots from the stressed plants were cut off at the end of the salt trial and cultured rather than risking loss of the genotype through the death of the mother plant during the recovery phase. Such shoots grew slowly in culture, developed unusual amounts of callus on their leaves, and often carried internal contaminants. This strategy did however save some genotypes which would have otherwise been lost.

Good cultures were obtained from new, rapidly growing shoots from healthy plants. These were surface sterilised with 1-2% sodium hypochlorite, or, for very soft material, 4-6% calcium hypochlorite. At the beginning of the project, shoot

growth was initiated *in vitro* from nodal explants on an initiation medium (MS with 2.5 μM IBA, 3.7 μM BAP, 5.5 μM kinetin and 3% sucrose) but it was later found that equally good results were obtained by initiating explants on the standard shoot multiplication medium (MS with 1 μM BAP and 0.01 μM NAA with 3% sucrose). It was found that *E. camaldulensis* lines stabilised within 2 months of establishment of a shoot culture, but most eucalypt species required 4 months (to 12 months or more) to stabilise and give good shoot growth and elongation. The length of time required was surprising since the explants had been taken from mother plants only about 1-1.5 years old.

Table 1. Genotypic variation and change with time in culture, of root initiation of salt tolerant *E. halophila* and *E. spathulata*, ssp *spathulata* lines. The first trial was 6 months after culture initiation and the second 3 months later. The rooting medium was $^1/_2$MS with 1 μM IBA and the number of shoots in each treatment is shown in parenthesis.

	Rooting (%)	
Line	**Trial 1**	**Trial 2**
E.halophila		
HAO 504	60 (20)	85 (20)
HAO 505	90 (10)	80 (20)
HAO 509	55 (20)	53 (19)
HAO 512	81 (21)	100 (8)
	Mean 71.5	79.5
E.spathulata		
SPS 503	15 (20)	45(20)
SPS 504	100 (10)	100 (40)
SPS 505	52 (21)	100 (12)
SPS 516	90 (10)	87 (15)
SPS 523	40 (20)	90 (90)
SPS 529	72 (18)	85 (13)
	Mean 61.5	84.5

When shoot growth was adequate and reproducible over sequential subcultures a few lines of each species were tested for root initiation (Table 2). The standard root initiation medium was $^1/_2$ MS major minerals, minor minerals, vitamins and growth factors with 2% sucrose and varying levels of IBA. Cultures were kept on auxin media in the dark for 1 week before exposure to normal lighted culture conditions (16 h photoperiod c. 28 μmol m^{-2} s^{-1}). If necessary experiments were then conducted to optimise rooting, and all the lines for that species tested.

In our experience, given a relatively low output of each line, commercial laboratories prefer to use one medium for all lines of a species even though higher rooting percentages might be obtained by using individual media for particular genotypes. There was considerable genotypic variation in rooting ability within a species, and rooting percentages often increased with time in culture (Table 1). Where possible, sufficient salt tolerant selections were made to allow discard of those genotypes which did not establish, multiply well or produce acceptable rooting percentages.

Table 2. Response *in vitro* of lines of eucalypt species selected for high tolerance of saline waterlogging. The standard shoot multiplication medium was MS with 1 μM BAP and 0.01 μM NAA with 3% sucrose. Root intiation medium was $^1/_2$ MS major minerals, minor minerals, vitamins and growth factors with 2% sucrose and IBA levels as described.

Shoot multiplication	Root initiation
E. calycogona	
5 of 6 lines set up established and grew well in standard medium. Short stabilisation time.	0, 1, 5, 10 μM IBA tested (20 shoots per treatment) 1 μM IBA optimal. 5 lines gave rooting percentages from 20-90%.
E. comitae-vallis	
1 line; good shoot growth in standard medium.	Around 95% rooting in medium with 1, 5 or 10 μM IBA but less basal callus at 1 μM IBA. (25 shoots per treatment)
E. halophila	
11 of 14 lines set up established and grew well in standard medium. 3 established but grew poorly.	0, 1, 5, 10 μM IBA tested on 5 lines, 10-32 shoots per treatment. Best rooting % on media with auxin but little difference between auxin levels. Less basal callus with 1 μM IBA. 11 lines tested on 1 μM IBA gave 20-85% rooting. (10-30 shoots per treatment)
E. kondininensis	
5 of 7 lines set up established on standard medium. In some lines shoots were initially very short but height improved in stabilised cultures. 1 line required 1-4 weeks etiolation to produce shoots suitable for rooting.	0, 1, 5, 10 μM IBA tested for one line best rooting (66%) at 1 μM IBA. 2 out of 3 lines etiolated showed no improvement in rooting, one etoilated line improved from 63% to 86%. (No. of shoots per treatment = 10 - 30)

Shoot multiplication	Root initiation
E. kumarlensis	
2 of 3 lines set up established and showed low-adequate growth.	not tested
E. lesoufii	
1 line, did not establish.	not tested
E. myriadina	
1 line set up and showed adequate growth in standard medium	Tested on 0, 1, 5, 10 μM IBA. 95% rooting on 1 μM IBA. (20 replicates per treatment, 1 line)
E. occidentalis	
22 lines set up. Shoots initially small and compact and produced callus on leaves. Cultures took up to 12 months to stabilise.	Rooting tested on 0, 1, 5, 10 μM IBA. Little difference between 1, 5 and 10μM IBA (3 lines, 25 shoots per treatment). 7 lines tested at 5 μM IBA showed from 40-99% rooting (12-199 shoots per treatment).
E. platycorys	
1 line established and grew well in standard medium	1, 5, 10 μM IBA tested with 1 μM being best for rooting. When shoots were etiolated for 2-4 weeks before placing on 1 μM IBA, rooting increased from 52% to 95%. (20 - 25 shoots per treatment)
E. salicola	
4 lines set up established but gave very poor shoot growth	not tested
E. sargentii	
8 of 21 lines set up established and grew adequately. All initially had very compact growth, often with callus on leaves. Removal of NAA or a change from BAP to kinetin did not alleviate this. Slow and variable in time to stabilise (6 months to 2 years).	Rooting tested on 0, 1, 5, 10 μM IBA, little difference between 1, 5 and 10 μM IBA. On 5 μM IBA 24 - 100% rooting (10-25 shoots per treatment, 4 lines)
E. spathulata	
20 lines established and grew well on standard medium. Some callus on leaves but it was not detrimental	Rooting percentages 15-100% on 5 μM IBA (9 lines tested, 40 shoots per treatment)

Shoot multiplication	Root initiation
E. yilgarnensis	
5 of 6 lines established. Most grew and multiplied slowly. Shoots were short and had soft brown callus at the base.	On 5 μM IBA 33% rooting; on 10 μM IBA 25% rooting. (1 line tested 20-40 shoots per rep)
E. wandoo	
14 lines established. A test of BAP, kinetin and zeatin at 0.5, 1, 5, 10 μM showed kinetin at 5 μM plus NAA 1 μM to be optimum. After a year in culture a further test showed BAP at 1 μM to give better growth than kinetin (10 lines tested).	Full MS with 0, 5, 10, 25 μM IBA tested, 5 μM optimal, 20-100% rooting (8 lines tested). Etiolated shoots have better rooting than normal ones in a few lines.

It was found that no species grew as well, or stabilised as quickly *in vitro* as *E. camaldulensis* for which published methods were available (Hartney 1982; Hartney & Barker 1983). A wide range of species was selected to include species with different growth forms from a range of environmental tolerances, and for different end products; firewood, poles, oil, windbreaks, and pulp (Table 2). The most desirable species were those which had several uses. These were *E. camaldulensis, E. occidentalis, E. halophila* and *E. sargentii*. Unfortunately *E. sargentii* and *E. occidentalis* initially had a small compact growth form *in vitro*. Although some lines eventually produced elongated shoots in culture, this appeared to be a result of culture stabilisation rather than any culture or media manipulation and it could not be induced in all cultures. For some species, etiolation for 2-4 weeks resulted in long shoots with improved rooting. This improvement was lost if shoots were allowed to green and harden in the light before transfer to auxin medium. Keeping the shoot cultures in the dark, cutting off the etiolated shoots at the optimum time, and handling these brittle, delicate shoots proved an unsuitable technique for commercial use.

Stabilised shoot cultures were passed to Plantex (Australia) and to Alcoa (Australia) who produced plants for field trials and commercial sales.

Glasshouse and field trials of salt tolerant eucalypts

The ability of the salt tolerant clones to withstand saline conditions has been tested both using glasshouse pot trials and in the field. There are various possibilities for use as 'control' plants in such trials, but it was decided to use seedlings from the same seed provenances as the selected clones. These seedling provenances usually exhibited salt tolerance well above the average for the species. In a glasshouse trial of *E. camaldulensis*, pots were freely drained but subjected to increases of 50 mM NaCl each week until a level of 1200 mM NaCl had been reached. In all cases the clones showed a higher percentage of survival than the seedlings (Bell *et al.* 1994).

Comparisons of survival and growth rates of clones and seedlings in the field is complicated by the fact that even in an area which is visually uniform, there may be significant variations in soil salinity. Consequently the most valid comparisons can be made when the soil in the vicinity of each tree is sampled. One such trial has been in place since 1989 at Narrogin in Western Australia and included *E. camaldulensis* clones and provenance matched seedlings. Clonal plants are surviving in areas of high salinity where seedlings have died, and in regions of lower salinity, the clones are generally growing faster than the seedlings (Bell *et al.* 1994).

In a two month establishment trial of *E. spathulata* near Mount Barker, Western Australia, in areas where soil conductivity was below 150 mSm^{-1} there was a dramatic difference in survival between the salt tolerant clones (73%) compared with the provenance-matched seedlings (8%). In areas with higher salinity no seedlings survived but there were a few representatives of two of the three clones (Bell *et al.* 1994). Trials of clones and seedlings have been set up in saline sites around Australia and overseas (McComb *et al.* 1989; Bell *et al.* 1994). It has been found that in conditions severe enough to reduce the survival of the seedlings the performance of the best clonal line was always better than that of the seedlings.

A criticism that is sometimes levelled at tissue cultured trees is that they have fibrous superficial roots, lack a taproot and may be vulnerable to wind throw. There are little hard data to support these assertions and experiments with *E. camaldulensis* have shown that the criticisms are unfounded. *E. camaldulensis* clones and seedlings were grown for 9 months in 200 L drums before the soil was washed away to expose the roots. Both clones and seedlings had developed a system of 5-6 large sinker roots and the distribution of the roots and the total root length was not different in the two types of plants. The clonal lines each had a consistent recognisable root architecture. The variations fell within the range seen amongst the seedlings from the same provenance and were not a result of tissue culture per se (Bell *et al.* 1993).

Field trials of a number of species are in place and will need to be assessed over a period of years. In addition to testing for level of salt tolerance, the field trial plants have been used to quantify difference in water usage by clonal lines (Marshall, J.K & Bell, D. pers. comm) and in insect herbivory (Wallington 1992). The rights to use of the clonal lines have been purchased by Ny Pa (Australia), 122 Grant Avenue, Toorak Gardens, South Australia 5065.

Eucalypts important in plantations

Eldridge *et al.* (1993) consider that the ten most important eucalypts in the world for wood production are *E. grandis, E. camaldulensis, E. tereticornis* and *E. globulus* (these four are by far the most important), followed by *E. urophylla, E. viminalis, E. saligna, E. deglupta* and *E. exserta.* Tying for tenth place are *E. citriodoria, E. paniculata,* and *E. robusta.* All these species are in the

Symphyomyrtus, except for *E. globulus* which is in Corymbia. In this chapter we report in detail on the considerable research into *in vitro* techniques for *E. grandis, E. camaldulensis, E. tereticornis* and *E. globulus* and more briefly on the other widely-planted species. It is surprising how little work there is for some of the species that are extensively used in plantations. There appear to be no reports on *E. exserta* or *E. paniculata*, and very few on the remaining species.

Three major problems recur when dealing with the tissue culture of eucalypts. These are culture stabilisation, clonal variation and root induction. There are problems in interpretation and further application of some of the published information on tissue culture of eucalypts because of the frequent failure of authors to include (even an estimate of) the age of the original explants, the length of time cultures were maintained, and the number of genotypes used to obtain the reported results. When data are given for a large number of clones, it is clear that there may be a wide range of ability for multiplication and rooting *in vitro* and that the percentage of genotypes with both good multiplication and rooting may be as low as 5-10% (Willyams *et al.* 1992a, b)

E. grandis

E. grandis is probably the most widely planted of the eucalypts throughout the world (Eldridge *et al.* 1993). In Argentina, Brazil and South Africa it is the major plantation species. It is valued for its form, fast growth rates, pest resistance, pulping quality and coppicing ability (Malan 1988). Extensive work has been done on this species to develop clonal seed orchards, hybridisation techniques and vegetative propagation through cuttings (Campinhos & Ikemori 1977; van Wyk 1990; Eldridge *et al.* 1993).

It was the first eucalypt species to be tissue cultured for forestry purposes and work was initiated in the early 1970s by workers such as de Fossard *et al.* (1974b). Early work concentrated on developing conditions suitable for shoot culture, then root induction and more recently somatic embryogenesis. Surface sterilisation of explants from mature trees in the field was difficult due to contamination and the production of phenolics after culture initiation (Cresswell & Nitsch 1975; Holden & Paton 1981). These problems can be largely overcome by collecting material at particular times of the year, selection of appropriate explants and varying the sterilisation procedure (eg Holden & Paton 1981).

Micropropagation

Most reports for *E. grandis* utilise juvenile material from aseptic seedlings or surface sterilised seedling explants (de Fossard 1974; Cresswell & de Fossard 1974; Goncalves *et al.* 1979; Lakshimi Sita 1982; Hartney & Barker 1983; Furze & Cresswell 1985; Rao & Venkateswara 1985; Lubrano 1991). Media and conditions that have been developed for juvenile explants have had some application to mature material. Five year old trees have been cultured by a number of researchers (Durand-Cresswell & Nitsch 1977; Lakshmi Sita & Rani 1985) while other authors have reported limited success with mature trees of unspecified

age (Furze & Cresswell 1985; Rao & Venkateswara 1985). It was the first species in which the content of growth regulators was found to correlate with rooting ability *in vitro* and *in vivo* (Paton 1983).

The media requirements are similar to those reported for other eucalypts (McComb & Bennett 1986; Le Roux & van Staden 1991) but MacRae and van Staden (1990) reported that when gelrite was used to solidify media in preference to agar, there was improved shoot growth and rooting of cultures from 3 month old seedlings. Rooting and establishment in soil was found to be better from a medium with no hormones and 1% activated charcoal, than from media with auxin, for a *E. grandis* x *urophylla* hybrid (Jones & van Staden 1994).

Callus cultures and embryogenesis

E. grandis callus was not difficult to induce (de Fossard *et al.* 1974b; Goncalves *et al.* 1979; Raghavan 1986). Warrag *et al.*, (1991) reported regenerating plantlets from hypcotyl callus of *E. grandis* hybrids. Raghavan, (1986) and Laine and David, (1994) have reported regeneration off callus from older explants such as coppice. Successful regeneration from these cultures of mature tissues may have been partly due to genotypic variations, but also due to the 3 year stabilisation period.

Several workers have reported development of somatic embryos from *E. grandis* callus (Lakshimi Sita 1982; Watt *et al.* 1991). This has been observed from juvenile material and there has been some success in regenerating whole plants.

Genetic engineering

There is considerable interest in incorporating foreign genes into eucalypts. This may serve two purposes; to introduce desirable genes to aid productivity or to increase adventitious root production through introducing root induction genes. MacRae and Van Staden (1993) obtained rooted shoots of *E. grandis* using *Agrobacterium rhizogenes* transformation of *in vitro* shoots. The level of rooting was no higher than that achieved using an auxin in the medium. It remains to be seen whether the clonal variation seen in adventitious root production can be overcome using this approach.

E. globulus

E. globulus was initially planted overseas as an ornamental but is now widely planted for pulpwood. For some time it was the main plantation species outside Australia but is now probably not as important as *E. grandis* and *E. tereticornis*. In Australia it is becoming increasingly important in plantation development because of its high pulping quality and fast growth rates (De Little *et al.* 1992; CALM 1993). Research on this species as a plantation timber has concentrated on provenance variation (Eldridge *et al.* 1993), hybridisation for increased productivity and cold tolerance (De Little *et al.* 1992) and clonal variation and site specificity (Borralho *et al.* 1992).

Micropropagation

Most of the work that has been done on *E. globulus* has concentrated on micropropagation. This, as with most eucalypts, has been most successful with juvenile explants (Hartney & Barker 1983; Kuzminsky & Lubrano 1991; Willyams *et al.* 1992) but there have also been some success with 3 year old trees (Salinero 1983) or trees old enough to be assessed for desirable characteristics such as growth rate and pulping quality (Trindade *et al.* 1990; Bennett *et al.* 1992b; Bennett *et al.* 1994). Trindade *et al.* (1990) obtained suitable explants from 12 year old trees by cutting off large branches and placing them in buckets of water in a growth chamber to induce epicormic shoots to sprout.

Media used by different researchers for this species are very similar, with MS as a basal medium and a low auxin:cytokinin ratio producing most effective shoot growth and multiplication (Trindade *et al.* 1990; Bennett *et al.* 1994). Bennett *et al.*, (1992b; 1994) reported that sustained shoot multiplication and growth could only be obtained when the type of cytokinin in the shoot multiplication medium was alternated with each passage. Kuzminsky and Lubrano (1991) found it was necessary to reduce the concentration of BAP from 2.22 μM for initiation to 0.13 μM for shoot multiplication.

Root induction, as with other eucalypts, is a major problem. Again media composition reported from different laboratories is very similar, with IBA being the most useful auxin for inducing roots (Trindade *et* al. 1990). Salinero (1983) found a 1 minute dip in IBA 4,900 μM followed by culture in medium without hormones gave higher rooting than inclusion of IBA in the medium. Bennett *et* al., (1994) found that the most effective rooting was obtained when shoots were transferred from multiplication medium containing kinetin rather than BAP, an effect also reported for *E. gunnii* (Curir *et al.* 1990). The plantlets from kinetin medium compared with those from BAP medium also had healthier shoots and higher survival when transferred to soil (Bennett *et al.* 1994). Work on changes of endogenous levels of IAA in hypocotyl tissues which produce adventitious roots, opens the way to a better understanding of the problem of rooting cuttings of E. *globulus in vivo* and *in vitro* (Barwani *et al.* 1994).

Callus and protoplast culture

There are several reports on callus production and organ regeneration for *E. globulus* (Oka *et al.* 1982; Chriqui *et al.* 1991). Successful regeneration has been achieved from hypocotyl or cotyledonary explants; callus from older explants has not regenerated. Chriqui *et al.*, (1991) reported best shoot regeneration on MS medium containing IAA and BAP in a 1:2.2 ratio.

Attempts at protoplast culture have not been successful. Teulieres and Boudet (1991) attempted to isolate *E. globulus* protoplasts but do not report requirements for their culture.

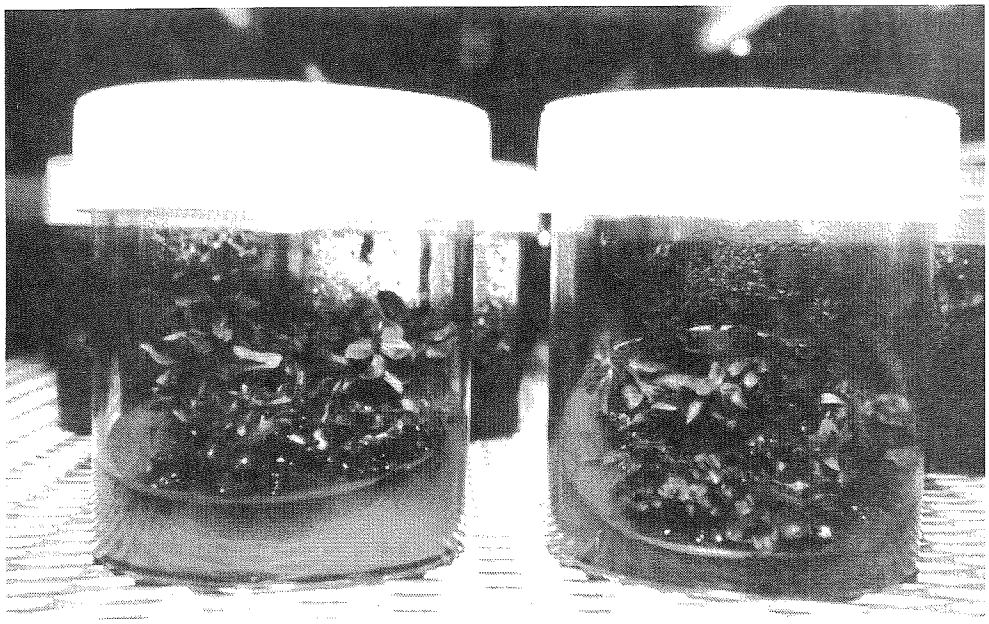

Figure 4: *Eucalyptus globulus* shoot cultures grown on MS medium with 2.5 μM BAP or 2.5 μM kinetin in alternate subcultures.

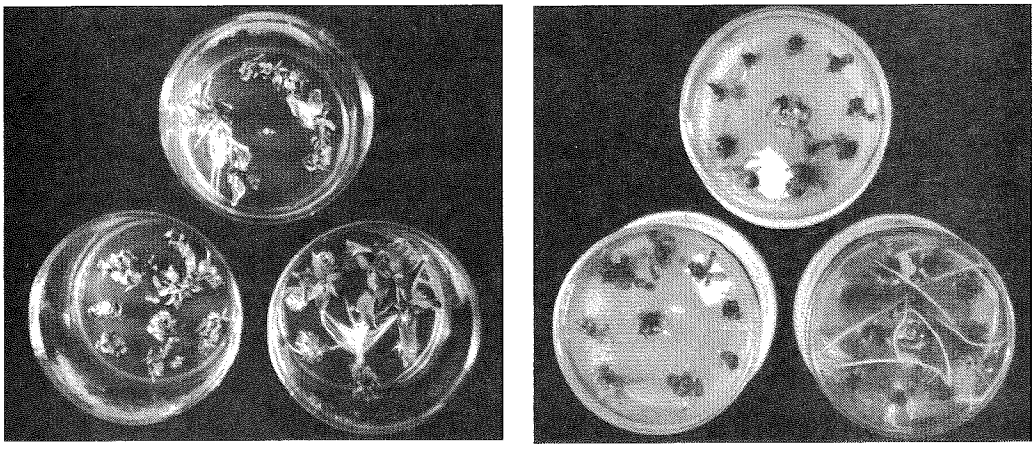

Figure 5: *Eucalyptus globulus* in tubs of rooting medium (A) from above, (B) from below. Shoots were taken from different multiplication media; with 2.5 μM kinetin (bottom right); 2.5 μM BAP (top); 1.25 μM kinetin and 1.25 μM BAP (bottom left). Note that best root formation is on shoots from media with kinetin alone, and that shoots from media containing BAP are dead or dying.

Genetic engineering

Chriqui *et al.* (1991) reported genetic transformation of *E. globulus* using *Agrobacterium tumefaciens* and *A. rhizogenes* mediators. The level of transformation was low and the strain of bacterium was very important. Teuliers *et al.* (1994) have found a promising approach, the biolistic treatment of 6-day old seedlings followed by regeneration through organogenesis.

E. tereticornis

The greatest productivity of *E. tereticornis* probably comes from hybrids that may have originated from several different sources (Eldridge *et al.* 1993). The hybrid 'Mysore gum' is the main eucalypt planted in India, but there are also substantial areas planted in Brazil. It is fast growing and produces timber and paper pulp as well as being used in amenity plantings. Measurement of height, basal diameter and biomass of micropropagated *E. tereticornis* after both 1 year's and 3 year's growth in the field were significantly greater than for seedlings (Gupta *et al.* 1991).

Micropropagation

Micropropagation from both seedlings and mature trees has been reported for *E. tereticornis* (Ilahi & Jamal 1987; Rao 1988; Das & Mitra 1990). Das and Mitra (1990) reported a high rooting percentage (60-80%) from cultures from 8-10 year old trees but gave no indication of the number of genotypes for which this was obtained. Only low rooting (12%) was obtained from explants from older trees (Rao 1988).

Callus and organogenesis

Subbaiah and Minocha (1990) reported the regeneration of shoots from hypocotyl segments and the subsequent multiplication and rooting. Venkateswara *et al.* (1986) grew callus cultures to examine the phytochemical constituents of the cells.

E. camaldulensis

The popularity and success of *E. camaldulensis* in plantations has been attributed to its ability to produce wood under less favourable conditions than the previous three species. *E. camaldulensis* can be productive on infertile and dry sites and show tolerance to drought, high temperature and salt. There have been several programs involving the use of tissue culture of *E. camaldulensis* (Hartney 1982; McComb *et al.* 1989) which have been successful because selected juvenile material was used and *E. camadulensis* as a whole responds very well in tissue culture (Hartney & Barker 1983; McComb *et al.* 1989).

Micropropagation

First reports of micropropagation of *E. camaldulensis* (Hartney 1982; Hartney & Barker 1983) used explants up to 6 months old. For this species both shoot multiplication and root induction are prolific when juvenile material is used and commercial nurseries in Australia produce clones of salt tolerant individuals of this species. Micropropagation of mature trees is achieved by use of basal coppice. When shoots are cultured in the dark for several months, on medium with auxin and activated charcoal, masses of nodular meristems may proliferate from the nodes. These may be induced to sprout shoots by subsequent exposure to light and GA3 (Boxus *et al.* 1991; Ossor & Boxus 1992). Root development of micropropagated plants was shown to be similar to that of seedlings in terms of both architecture and total length, after 9 months growth in the field (Bell *et al.*

1993). Cultures of *E. camaldulensis* are being used as models for trials of photoautotrophic growth (Kirdmanee *et al.* 1994).

Callus culture and genetic engineering

Regeneration from juvenile *E. camaldulensis* callus has been reported (Diallo & Duhoux 1984; Mullins *et al.* 1991). Leaves from cultured shoots also produce callus that will regenerate shoots (Muralidharan & Mascarenhas 1987). Mullins *et al.* (1991) investigated the possibility of genetic transformation of this species and others using *Agrobacterium*. *E. camaldulensis* was conducive to this treatment and regeneration was of high frequency, making this species one which would be ideal to further experimentation on genetic transformation.

E. urophylla

In *E. urophylla*, using nodal or shoot tip explants shoot multiplication was achieved with 10 μM BAP and shoots rooted in medium with no hormones (Goncalves 1980; Goncalves *et al.* 1979). However Grattapaglia *et al.* (1990) used IBA or IAA to induce roots on shoots *in vitro*. Rooting on medium with no hormones and 1% activated charcoal was effective for a *E. grandis* x *urophylla* hybrid (Jones & van Staden 1994). A technique using fungus on a mat of non-woven material has been described for *in vitro* inoculation of *E. urophylla* x *grandis* with the mycorrhizal fungus *Pisolithus tinctorius* (Galli *et al.* 1992).

A high level of shoot regeneration from hypocotyl explants has been reported by Tibok *et al.* (1994). Dos Santos *et al.* (1990) established suspension cultures from calli of seedling explants for protoplast extraction but the stage of subsequent growth reached by the protoplasts is not clear.

E. viminalis

Mehra Palta (1982) described in detail a complete cycle of micropropagation for *E. nova-anglica* and commented that preliminary studies showed that similar responses were obtained with *E. viminalis*. However no data on *E. viminalis* were given in this paper and it is not clear whether micropropagated clones of this species were established in soil.

Callus of *E. viminalis* has been used for studies of how patterns of peroxidase isozymes change with changes of growth regulators in the medium (Ishii 1982). The hybrid *E. viminalis* x *dalrympleana* produced in France was used in isolated root cultures and the production of nodules, was noted (Tripathi *et al.* 1983). In the presence of 4.9 μM IBA and 1.32 μM BAP (or, in addition 0.93 μM kinetin), the nodules developed into leafy shoots. This possible route to cloning does not appear to have been followed up successfully by further work on this hybrid or in other eucalypt species.

E. saligna

Regeneration of shoots from cultured hypocotyls has been reported by Tibok *et al.* (1994), and protoplasts were successfully extracted from suspension cells by Dos Santos *et al.* (1990). The most significant *in vitro* research on this species has been

done by the Oji Paper Company in Kameyama, Japan (Ito *et al. 1990*, Kawazu *et al. 1990*). They have succeeded in regeneration of plants from protoplasts and in genetic transformation of the species. Small clumps of multiple shoots were grown from shoot-tips in liquid B5 medium with 0.11 μM NAA and 2.02 μM N-(2-chloro-4-pyridyl)-N'-phenylurea (4 CPPU) in a rotated tube. Protoplasts isolated from the shoot primordia regenerated when co-cultured with kenaf (*Hibiscus cannabinus*) protoplasts which grow to callus but do not regenerate shoots. Eucalypt protoplasts developed into callus, then a mass of primordia which were also cultured in rotating liquid medium. Plants were successfully established in the field.

In further experiments, the Oji group found that *E. saligna* protoplasts take up foreign DNA after electroporation. Genticin resistance was initially used as a marker for transformed colonies but as the eucalypt cells were very sensitive to this antibiotic, neomycin phosphotranferase II (npt-II) was a better selectable marker. Southern blot analysis of regenerated shoots showed incorporation of the BT gene for insect resistance, and histochemical analysis indicated that transformed shoots had various levels of GUS (ß-glucuronidase) activity.

E. deglupta

There is little information on *E. deglupta* tissue culture. Shoot tips and nodes from axenic seedlings have been rooted *in vitro* (Cresswell & de Fossard 1974) and there is a brief report of a mass of shoot primordia being induced from shoot tips cultured (presumably) in the same type of rotating liquid cultures as described for *E. saligna* (Doi *et al. 1987*).

E. robusta

Goncalves (1975) used nodal pieces of *E. robusta* to induce shoot proliferation and subsequent rooting *in vitro*. Nodular clusters of meristems have been induced in the species similar to those described above for *E. camaldulensis* (Boxus *et al.* 1991). Seedling callus from the species has also been used to study the relationship between exogenous growth regulators and the production of flavanols *in vitro* (Samejima *et al.* 1982) and volatile compounds (Yamaguchi & Fukuzumi 1982). No monoterpenes were detected but there was a number of fatty acids, aldehydes, ketones and alcohols.

E. citriodora

E. citriodora is not planted widely in plantations except in India, where, apart from its use as timber it is used for rayon manufacture and extraction of lemon scented oils (citronellal and citronellol). Plantations were established in the 1930s and the species has been extensively studied *in vitro* by Indian scientists. One of the earliest reports of tissue culture of a eucalypt was of lignotuber explants which were induced to form callus then regenerate shoots some of which sprouted (Aneja & Atal 1969). Production of multiple shoots, rooting and establishment of plantlets in soil was achieved first using seedling explants (Lakshmi Sita & Vaidyanathan 1979; Grewal *et al.* 1980), then with material from crowns of mature trees (Grewal *et al.* 1980; Gupta *et al.* 1981; Mascarenhas *et al.* 1982).

Regeneration of shoots from callus of seedling origin was achieved by Lakshmi Sita (1979) but explants from plants a year or more old were difficult to regenerate.

Large numbers of embryos can be induced on seedling callus of *E. citriodora* (Lakshmi Sita 1982) but they proved difficult to germinate. However using direct embryogenesis from mature zygotic embryos or cotyledons, and repetitive embryogenesis Muralidharan and Mascarenhas (1987) and Muralidharan *et al.* (1989) were able to obtain rooted plantlets from 50% of the adventitious embryos.

Protoplasts of *E. citriodora* cotyledons have been regenerated to colonies of 60-80 cells (Dos Santos *et al.* 1990).

Tissue cultured plants show more uniform growth than seedlings and have similar levels of citronellal and citronellol to the parent tree within 1-2 years, whereas seedlings require 3-4 years growth to attain this level (Gupta *et al.* 1991).

Other species of eucalypts

The excellent recent review of Le Roux and van Staden (1991) tabulates the results of tissue culture for all eucalypt species and it is unnecessary to repeat that information. Only publications not available for the Le Roux and van Staden (1991) review are listed here. For convenience, we have used a very similar format to that used by Le Roux and van Staden (see Table 3 at the end of this chapter).

Conclusions: the future

Production gains for all the end uses of eucalypts will be increased by effectively coupling vegetative propagation to conventional breeding programmes. At present there are relatively few species for which micropropagation is sufficiently cost effective to be routinely used for production of plants for large scale field plantings. Strategies to rejuvenate plants before culture initiation and to overcome problems of internal contamination are now well known. However for commercial production, lines must show at least 70–80% rooting and 90% survival on transfer to soil. The physiology and biochemistry of culture stabilisation and of the control of high shoot multiplication and adventitious rooting needs much more research. It has been suggested that 80% of time should be spent on screening genotypes of élite trees to select those that perform well in culture, and 20% in optimising media for those good genotypes. This is a good strategy when there is a large number of élite trees form which to select, but in many cases there will only be a few élite trees available, or the selections will come from a narrow genetic base. 'Wastage' of élite genotypes will be reduced as in vitro techniques improve through knowledge of *in vitro* physiology rather than through empirical modification of media and growing conditions.

Automation is expected to reduce the cost of propagules from culture, and may take the form of automated preparation and handling of encapsulated embryos, or of robotic shoot subculture and transfer of plantlets to soil. The improvement of photoautotrophic growth systems in which sugar can be removed from the media will allow for easier involvement of robots with less danger of

contamination. It is possible that the most economic system to evolve might lie somewhere between conventional cuttings and micropropagation and be a type of photoautotrophic 'microhydroponic' growth system cropped robotically.

Genetic engineering of eucalypts appears feasible as success has been reported using electroporation of protoplasts, biolistics and *Agrobacterium tumefaciens* and *A. rhizogenes*. Except for flowering ornamentals and honey production, eucalypt products are of vegetative parts of the plant, and there may be less public disquiet about genetically engineered trees than over edible plants. However in Australia where plantations may be adjacent to natural stands of a species, it is essential that trees engineered for desirable characters of growth, or wood production etc, are also engineered so that they are both male and female sterile. Flow of foreign genes from genetically engineered trees, to the surrounding forest would be unacceptable.

Acknowledgments

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Table 3. Summary of media and *in vitro* reponses of *Eucalyptus* species. Media references are as shown and MS (Murashige and Skoog 1962); N&N (Nitsch and Nitsch 1967), B5 vitamins (Gamborg *et al.* 1968); Hoaglands (Janick 1986), WPM (Lloyd and McCown 1980); de Fossard (1977) vitamins (see Barker *et al.* 1977). Numbers in the first column indicate a sequence of stages.

Only data not included in Table 1 in the review by Le Roux and van Staden (1991) are included here.

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
	E. botryoides					
1	seedlings, shoot cultures, leaves,tips, nodes	morphogenic callus				Qin & Kirby 1992
	E. calycogona					
1	nodes from plants 1-$^1/_2$ years old	shoot mulitiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. camaldulensis					
1	*in vitro* shoots	nodular meristematic clusters	MS	5.6/30/7 agar (cultured in darkness)	4.9 μM IBA 2 g/L activated charcoal	Boxus *et al.* (1991) Ossor & Boxus (1992)
2	mersitematic nodules (1)	shoot elongation		" (in light 12 h/ darkness 12 h)	1.44 μM GA3 2 g/L activated charcoal	
3	*in vitro* shoots (2)	rooting	$^1/_2$MS	" (in light 12 h/ darkness 12 h)	no hormones	
	E. cinerea					
1	seedlings, shoot cultures, leaves, tips, nodes	morphogenic callus			not given in detail	Qin & Kirby (1992)

	E. comitae-vallis					
1	nodes from plants 1-1$^1/_2$ years old	shoot mulitiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. dalrympleana					
	leaves from 3-6 month old plants	protoplasts				Teulieres *et al.* (1989a) Teulieres & Boudet (1991)
	E. dalrympleana					
1	nodes from sterile seedlings shoots		MS	5.7/30%/7 agar	2.22 μM BAP 0.05 μM NAA	Kuzminsky & Lubrano (1991)
2	*in vitro* shoots (1)	shoot multiplication	"	"	0.14 - 0.44 μM BAP 0.05 μM NAA	
3	*in vitro* shoots (2)	rooting	$^1/_2$Knops (1865)	"	4.9 μM IBA	
4	rooted *in vitro* shoots (3)	root elongation	"	"	no hormones 10 g/L activated charcoal	
	E. delegatensis					
1	leaves from glasshouse plants	protoplasts				Teulieres *et al.* (1989a) Teulieres & Boudet (1991)
	E. dunnii					
1	seedlings, shoot cultures, leaves, tips, nodes	morphogenic callus	not given in detail			Qin & Kirby (1992)
	E. ficifolia					
1	leaves from glasshouse plants		protoplasts			Teulieres *et al.* (1989a) Teulieres & Boudet (1991)

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
	E. globulus					
1	epicormic or coppice shoots from 12 year-old trees	shoots	de Fossard (1974)	-/-/5 agar	1.1 - 2.2 μM BAP or Kinetin	Trindade *et al.* (1990)
2	*in vitro* shoots (1)	shoot multiplication	"	"	1.1 - 2.2 μM BAP and 0.5 μM IBA	
3	*in vitro* shoots (2)	rooting	"	"	IBA (conc not given)	
	E. globulus					
1	1-2 week old hypocotyls	shoots from callus	MS	-/-/-	0.08 μM BAP and 0.005 - 5.4 μM NAA or 0.91 μM TDZ	Chriqui *et al.* (1991)
2	12 day old hypocotyls (with Agrobacterium)	rhizogenic callus & tumorous callus (few transformed cells)		-/-/-		
	E. globulus					
1	nodes from sterile seedings	shoots	MS	5.7/30/7 agar	2.22 μM BAP 0.05 μM NAA	Kuzminsky & Lubrano (1991)
2	*in vitro* shoots (1)	shoot multiplication	"	"	0.14 - 0.44 μM BAP 0.05 μM NAA	
3	*in vitro* shoots (2)	rooting	$^1/_2$ Knops (1865)	"	4.9 μM IBA	
4	rooted *in vitro* shoots (3)	root elongation	"	"	no hormones 10g/L activated charcoal	

	E. globulus					
1	shoot tips from aseptic seedlings	shoot multiplication	MS	-/-/-	2.28 μM zeatin and 0.29 μM IAA	Willyams *et al.* (1992b)
2	seedling shoot tips as above or *in vitro* shoots (1)	rooting	WPM	-/-/-	94 μM IBA 2 days then no hormones	
	E. globulus					
1	nodes from seedlings, or coppice of 4-5 year old trees	shoot initiation and multiplication	MS	5 .8/20/2.5 agar and 2.5 gelrite	1.25 μM NAA and 2.5 μM BAP alternated with 2.5 μM kinetin	Bennett *et al.* 1994
2	*in vitro* shoots (1)	rooting	$^1/_4$MS macro nutrients full strength micro nutrients	"	10 μM IBA	
	E. globulus					
1	6-day old seedlings (biolistic treatment)	transformed cells followed by shoot regeneration				Teulieres *et al.* (1994)
	E. grandis					
1	shoots from sterile seedlings	shoot multiplication	MS	5.7/30/4 agar	0.05 μM NAA and 40 μM BAP	Watt *et al.*. (1991)
2	leaves from *in vitro* shoots (1)	callus	"	"	4.5 μM 2,4-D	
3	embryonic callus (2)	plantlets"	"		0.05 μM NAA and 0.44 μM BAP and 0.29 μM GA3 and 4 g/L activated charcoal	
	E. grandis					
1	shoots from aseptic seedlings	shoot multiplication	MS, de Fossard (1977) vitamins	-/-/-	2.22 μM BAP	Lubrano (1991)
2	*in vitro* shoots (1)	shoot elongation	"	"	0.22 μM BAP (darkness 3 weeks)	
3	elongated shoots (2)	rooting	$^1/_2$Knops (1865)	"	5.37 μM NAA	

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
	E. grandis					
1	nodes from 3 month old seedlings	shoot multiplication	MS	5.8/30/2 gelrite and 0.05 μM NAA	0.89 μM BAP	MacRae and van Staden (1990)
	E. grandis					
1	shoots from aseptic seedlings	shoots with transformed roots (Agrobacterium rhizogenes)	MS	5.8/30/2 gelrite		MacRae and van Staden (1993)
	E. grandis					
1	nodes from coppice shoots	callus	Laine & David (1994	5.7/20/2.5 phytagel	8.9 -22.2 μM BAP and 2.69 - 13.42 μM NAA) or 39.2 μM zeatin and 2.69 μM NAA	Laine & David (1994)
2	callus from shoot cultures (1)	shoot regeneration	"	"		
3	shoots (2)	rooting	"	"	with or without IBA	
	E. grandis hybrids					
1	hypocotyl explants and several types of explants from 5 year old trees	? callus then shoot regeneration	MS B5 vitamins 200 mg/L glutamine	5.5/45/6.5 agar	4.60 μM kinetin 21.48 μM NAA 10% coconut milk	Warrag *et al.* (1991)
	E. grandis x urophylla					
1	nodes from hedged cuttings	shoot growth	$^1/_2$MS	5.8/20/2.0 gelrite	0.44 μM BAP	Jones & van Staden, (1994)

2	*in vitro* shoots (1)	shoot multiplication	"	"	0.88 μM BAP and 0.05 μM NAA	
3	*in vitro* shoots (2)	rooting	"	"	no hormones 10 g/Lactivated charcoal	
	E. graniticola					
1	*in vitro* shoot apices encapsulated	cryopreservation				Touchell & Dixon (1994)
	E. gunnii					
1	leaves from 3-6 month old plants	protoplasts				Teuliers *et al.* (1989a)
	E. gunnii					
1	leaves from glasshouse plants	callus	MS	-/-/7.5 agar	9 μM 2,4-D	Teuliers *et al.* 1989(b)
2	friable callus (1)	cell suspensions	"	-/-/0	0.44 μM BAP 13.4 μM then 6.7 μM 2,4-D	
3	cell suspensions (2)	protoplast transformation using electroporation				Teuliers *et al.* (1991)
4	cell suspensions (2)	protoplast culture to micro colonies				Teulieres & Boudet (1991)
	E. gunnii					
1	2-week old cotyledons	shoots from callus	MS	-/-/-	1 μM IAA + 2.2 μM BAP	Chriqui *et al.* (1991)
2	*in vitro* shoots (1)	shoot elongation	"		no hormones	
3	*in vitro* shoots (2)	shoot multiplication	"		0.9 μM BAP + 0.05 μM NAA	
4	*in vitro* shoots (3)	rooting	"		15 μM IBA	

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
1	seedling cotyledons or hypocotyls (24 days old) (with *Agrobacterium*)					
2	transformed roots (1)	rhizogenic callus or	"			
		tumorous callus (no solid tranformed shoots)	"			
		isolated root growth	"		no hormones	
	E. gunnii					
1	*in vitro* shoot tips	cryopreservation				Monod *et al.* (1992)
	E. gunnii x dalrympleana					
1	leaves from 3-6 month old plants	protoplasts - best yield from *in vitro* leaves				Teulieres *et al.* (1989a) Teulieres & Boudet (1991)
2	leaves from *in vitro* shoots					
	E. gunnii x globulus					
1	leaves from 3-6 month old plants	protoplasts				Teulieres *et al.* (1989a) Teulieres & Boudet (1991)
	E. gunnii x ovata					
1	leaves from 3-6 month old plants	protoplasts				Teuliers *et al.* (1989a) Teulieres & Boudet (1991)
	E. gunnii x viminalis					
1	leaves from glasshouse plants	protoplasts				Teuliers *et al.* (1989a) Teulieres & Boudet (1991)

	E. halophila					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. kondininensis					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. kumarlensis					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
	E. marginata					
1	nodes from mature trees	shoot multiplication	MS	5.7/20/10	2.5 μM BAP and 2.5 μM NAA	Bennett *et al.* (1986, 1992a, 1993)
2	*in vitro* shoots (1)	rooting	$^1/_4$MS major and minor minerals $^1/_4$CaCl$_2$	5.7/20/8	10 μM IBA	
1	nodes from1-1$^1/_2$year old plants	shoot multiplication	MS	-/20/-	2.5 μM BAP and 1.25 μM NAA	McComb *et al.* (1990, 1994)
2	*in vitro* shoots (1)	rooting	$^1/_4$MS major minerals full micro $^1/_2$Fe	-/20/-	10 μM IBA	

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
	E. myriadena					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. nitens					
1	hypocotyls from seedlings 2-3 weeks old	shoot regeneration	MS	-/20/2 phytogel	NAA and TDZ (optimum concentrations not given)	Tibok *et al.* (1994)
	E. occidentalis					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS		5 μM IBA	
	E. ovata					
1	leaves from 3-6 month old plants (1991)	protoplasts				Teuliers *et al.* (1989a) Teulieres & Boudet
	E. parvifolia					
1	apices from shoots of 2 year-old trees	elongated shoots	Quiorin & Le Poivre (1977) macro elements de Fossard (1977) vitamins	5.7/30/7 agar	1 μM GA_3	Texier & Faucher (1986)
	E. pauciflora					
1	leaves from glasshouse plants	protoplasts				Teuliers *et al.* (1989a) Teulieres & Boudet (1991)

	E. perrineana					
1	young stems	callus	MS	-/30/9 agar	4.5 μM 2,4-D and 4.6 μM BAP 7% coconut milk	Furuya *et al.* (1987)
2	callus (1)	callus maintenance	"	"	4.4 μM BAP or 4.6 μM kinetin	Orihara *et al.* (1991)
3	callus (2)	suspension culture	"	-/30/0	4.4 μM BAP	Furuya *et al.* (1989) Ushiyama & Furuya (1989) Orihara *et al.* (1992)
	E. platycorys					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro shoots* (1)	rooting	$^1/_2$MS	"	1 μM IBA	
	E. radiata					
1	epicormic shoots nodes & tips from mature trees	shoot multiplication	N+N (1967)	-/-/7 agar	1 μM BAP and 1 μM kinetin and 8 μM IBA	
2	*in vitro* shoots (1)	v low % rooting	$^1/_4$ or $^1/_2$ MS	-/-/-	2.7 - 16.1 μM NAA or 2.4 - 14.8 μM IBA	
	E. radiata					
1	coppice and mature nodes	multiple shoots	MS	5.7/30/2 gelrite	BAP 0.5 mg/L and NAA 0.01 - 0.1 mg/L	Chang *et al.* (1992)
2	*in vitro* shoots (1)	elongated shoots	"	"	0.44 μM BAP & 0.05 - 0.54 μM NAA or 2.22 μM BAP & 0.5 μM NAA, and 0.29 - 2.9 μM GA_3 with 5 g/L activated charcoal for early subcultures	
3	*in vitro* shoots (2)	rooted shoots	$^1/_2$MS	3.5 - 4.5/10 - 20/6 gelrite	13.4 μM IBA (mature explants) 24.6 μM IBA (coppice explants)	

	Species & Explant	Result	Medium	pH/Sucrose/Gelling Agent (g/L) (g/L)	Growth Regulators	Reference
	E. regnans					
1	nodes from aseptic seedlings	shoot multiplication	MS	-/20/2.2 gelrite	2.28 μM zeatin and 0.27 μM NAA	Blomstedt *et al.* (1991) Willyams *et al.* (1992a)
2	*in vitro* shoots	rooting	Hoaglands or WPM	-/20/-	98 μM IBA 7 day pulse then no hormones	
	E. rudis					
1	seedlings, shoot cultures, leaves, tips, nodes	morphogenic callus			not given in detail	Qin & Kirby (1992)
	E. saligna					
1	hypocotyls from 2-3 week old seelings	shoot regeneration	MS	-/20/2 phytagel	NAA and TDZ (optimum concentration not given)	Tibok *et al.* (1994)
	E. sargentii					
1	nodes from plants 1-1 $^1/_2$ years old	shoot mulitiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	5 μM IBA	
	E. sideroxylon					
1	seedling shoots or nodes	direct rooting	$^1/_2$MS B_5 vitamins	5.8/20-60/6 agar	2.5 μM IBA + 2.5 μM NAA	Cheng *et al.*(1992)
	E. spathulata					
1	nodes from plants 1-1 $^1/_2$ years old	shoot mulitiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$MS	"	5 μM IBA	

E. tereticornis						
1	shoots from aseptic seedlings	shoot multiplication	B_5 and others	5.5/-/10 agar	2.22 μM BAP	Subbaiah & Minocha (1990)
2	*in vitro* shoots (1)	rooted shoots	Subbaiah & Minocha (1990)		2.46 μM IBA	
E. torelliana x citriodora						
1	nodal segments 8-year-old tree	shoots	MS	5.8/30/6 agar	8.9 μM BAP and 5.4 μM NAA	Kapoor & Chauhan (1992)
2	*in vitro* shoots (1)	shoot multiplication	"	"	8.9 μM BAP	
3	*in vitro* shoots (2)	rooting	1/5 or $^1/_2$ MS	"	0.49 μM IBA	
	E. urophylla					
1	3-week old seedlings	shoots, then roots	MS	-/-/-	BAP and NAA or IBA (conc not given)	Umboh *et al.* (1989)
	E. urophylla					
1	hypocotyls from seedlings 2-3 weeks old	shoot regeneration	MS	-/20/2 phytagel	NAA and BAP or TDZ (concentrations not specified)	Tibok *et al.* (1994)
	E. wandoo					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM NAA and 5 μM kinetin or 1 μM BAP	This review
2	*in vitro* shoots (1)	rooting	"	5.8/20/2.5 agar + 2.5 gelrite	5 μM IBA	
	E. yilgarnensis					
1	nodes from plants 1-1$^1/_2$ years old	shoot multiplication	MS	5.8/30/2.5 agar + 2.5 gelrite	1 μM BAP and 0.01 μM NAA	This review
2	*in vitro* shoots (1)	rooting	$^1/_2$ MS	5.8/20/2.5 agar + 2.5 gelrite	5 μM IBA	

References

Aneja, S. & Atal, C. (1969). Plantlet formation in tissue cultures from lignotubers of *Eucalyptus citriodora* Hook. Curr. Sci. 38: 69-70.

Barwani, O., Lewis, M., Brackpool, A. & Blakesby, D. (1994). Auxin and adventitious root initiation in *Eucalyptus globulus*. (Abstract) VIIIth Intern. Congress Plant Tissue and Cell Culture. Firenze (pp 216).

Barker, P.K., de Fossard, R.A. & Bourne, R.A. (1977). Progress towards clonal propagation of Eu*calyptus* species by tissue culture technique. Comb. Proc. Intern. Pl. Prop. Soc. 27: 546-556.

Bell, D.T., van der Moezel, P.G., Bennett, I.J., McComb, J.A., Wilkins, C.F., Marshall, S.C.B. & Morgan, A.L. (1993). Comparisons of clones and seedlings of *Eucalyptus camaldulensis*: Root shoot and leaf morphology of 9 month old plants grown in deep sand and sand over clay. For. Ecol. Manag. 57: 125-139.

Bell, D., McComb, J., van der Mozel, P., Bennett, I. & Kabay, E.D. (1994). Comparison of selected and cloned plantlets against seedlings for rehabilitation of saline and waterlogged discharge zones in Australian agricultural catchments. Aust. For. 57: 69-75.

Bennett, I.J. (1988). Use of Tissue Culture Techniques for the Improvement of *Eucalyptus marginata* Donn ex Sm. PhD thesis, Murdoch University.

Bennett, I. & McComb, J.A. (1982). Propagation of jarrah (*Eucalyptus marginata*) by organ and tissue culture. Aust. For. Res. 12: 121-127.

Bennett, I., Tonkin, C., Wroth, M., Davison, E., & McComb, J.A. (1986). A comparison of growth of seedlings and micropropagated *Eucalyptus marginata* (jarrah). I. Early growth to 2 years. For. Ecol. Manag. 14 : 1-12.

Bennett, I.J. McComb, J.A. & Bradley, J.S. (1992a). Testing the expression of resistance to insect attack: resistance of jarrah (*Eucalyptus marginata*, Donn ex Sm) to jarrah leaf miner (*Perthida glyphopa* Common). For. Ecol. Manag. 48: 99-105

Bennett, I.J., McComb, J.A., Tonkin, C.M. & McDavid D. (1992b). Effect of cytokinins on multipication and rooting of *Eucalyptus globulus* and other *Eucalyptus* species. IUFRO/ AFOCEL Conference Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species. Bordeaux, France Sept. 1992. 2: 195-202.

Bennett, I.J., McComb, J.A. & Tonkin, C.M. (1993) Inoculation of *Eucalyptus marginata* Donn. ex Sm. (jarrah) clones with *Phytophthora cinnamomi* Rands *in vitro* and under glasshouse conditions. For. Ecol. Manag. 57: 115-124.

Bennett, I.J., McComb, J.A. & McDavid D (1994). Alternating cytokinins in multiplication media stimulates *in vitro* shoot growth and rooting of *Eucalyptus globulus* Labill. Annals Bot. 74: 53-58

Blomstedt. C., Cameron, J., Whiteman, P. & Chandler, S. (1991). Micropropagation of juvenile *Eucalyptus regnans* (Mountain Ash). Aust. J. Bot. 39: 179-186.

Boland, D.J. Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A. & Turner, J.D. (1984). Forest Trees of Australia (4th Ed). Nelson & CSIRO Adelaide.

Borralho, N.M.G., Almeida, I.M. & Cotterill, P.P. (1992). Genetic control of growth of young *Eucalyptus globulus* clones in Portugal. Silvae Genetica 41: 100-105.

Boxus, P., Terzi J., Lievens, C., Pylyser, M., Ngaboyamahina, P. & Duhem, K. (1991). Improvement and persectives of micropropagation techniques applied to some hot climate plants. Acta Hort. 289: 55-64.

Brooker, M.I.H. & Kleinig, D.A. (1990). Field Guide to Eucalypts: Volume 1 South-eastern Australia. Inkata Press, Melbourne.

Brooker, M.I.H. & Kleinig, D.A. (1990). Field Guide to Eucalypts: Volume 2 South-western and Southern Australia. Inkata Press, Melbourne.

Butcher, T.B., Stukely, M.J.C. & Chester, G.W. (1984). Genetic variation in resistance of *Pinus radiata* to *Phytophthora cinnamomi*. For. Ecol. Manag. 8: 197-220.

Cahill, D.M. & McComb, J.A. (1992). Changes in phenylalamine ammonia lyase activity, lignin and phenolic synthesis in the roots of *Eucalyptus calophylla* (field resistant) and *E. marginata* (susceptible) when infected with *Phytophthora cinnamomi*. Physiol. & Mol. Pl. Pathol. 40: 315-332.

Cahill, D.M. Bennett, I.J. & McComb, J.A. (1991) Resistance of micropropagated *Eucalyptus marginata* to *Phytophthora cinnamoni* Plant Dis. 76: 630-632.

Cahill, D. Bennett, I. & McComb, J. (1993). Mechanisms of resistance to *Phytophthora cinnamoni* in clonal micropropagated *Eucalyptus marginata*. Pl. Path. 42: 865-872.

CALM 1993 Annual Report 1992 to 1993. The Western Australian Department of Conservation and Land Management.

Campinhos, E. & Ikemori, Y.K. (1977). Tree improvement of *Eucalyptus* spp. Preliminary results. In 3rd World Consultation on Forest Tree Breeding, Canberra (pp 717-738).

Chang, S.H., Donald, D.G.M. & Jacobs, G. (1992). Micropropagation of *Eucalyptus radiata* ssp *radiata* using explants from mature and coppice material. S. Afr. For. J. 162: 43-47.

Cheng, B., Peterson, C.M. & Mitchell R.J. (1992). The role of sucrose, auxin and explant source on *in vitro* rooting of seedling explants of *Eucalyptus sideroxylon*. Plant Sci. 87: 207-214.

Chippendale, G.M. (1988). *Eucalyptus*. In: Flora of Australia: Volume 19 *Myrtaceae-Eucalyptus, Angophora*. Australian Government Publications Service, Canberra.

Chriqui, D., Adams, S., Caissard, J.C., Noin, M. & Azmi, A. (1991). Shoot regeneration and *Agrobacterium* mediated transformation of *Eucalyptus globulus* and *gunnii*. IUFRO Symposium on Intensive Forestry: The Role of Eucalypts: Durban Sept. 1991, pgs 70-79.

Clarke, S.A. (1957). Notes on the utilisation of *Eucalyptus*. Aust. For. 21: 40-42.

Cresswell, R.J. & de Fossard, R. (1974). Organ culture of *Eucalyptus grandis*. Aust. For. 37: 55-69.

Cresswell, R.J. & Nitsch, C. (1975). Organ culture of *Eucalyptus grandis*. L. Planta 125: 87-90.

Curir, P., van Sumere, C.F., Termini, A., Barthe, P., Marchesini, A. & Dolci, M. (1990). Flavonoid accumulation is correlated with adventitious roots formation in *Eucalyptus gunnii* Hook. micropropagated through axillary bud stimulation. Pl. Physiol. 92: 1148-1153.

Das, T. & Mitra, G.C. (1990). Micropropagation of *Eucalyptus tereticornis Smith*. Pl. Cell Tissue Organ Cult. 22: 95-103.

de Fossard, R.A. (1974). Tissue culture of *Eucalyptus*. Aust. For. 37: 43-54.

de Fossard, R.A. (1978a). Nuclear stocks, multiplication rates and economic considerations of tissue culture propagation of horticultural species. Proceedings of Symposium on Plant Tissue Culture, Peking (pp 439-447) Science Press, Peking.

de Fossard, R.A. (1978b). Tissue culture of *Eucalyptus ficifolia*. F. Muell. Proceedings Symposium on Plant Tissue Culture, Peking. (pp 425-438) Science Press, Peking.

de Fossard, R.A. & Bourne R.A. (1977). Clonal propagation of *Eucalyptus* by nodal culture. Third World Consultation of Forest Tree Breeding. Canberra, Australia pp 1023-1029.

de Fossard, R.A., Myint, A. & Lee, E.C.M. (1974a). A broad spectrum experiment with tobacco *Nicotiana tabacum* pith tissue callus. Physiol. Plant. 31: 125-130.

de Fossard, R.A., Nitsch, C., Cresswell, R.J. & Lee E.C.M. (1974b). Tissue and organ culture of *Eucalyptus*. N.Z. J. For. Sci. 4: 267-278.

de Fossard, R.A., Barker, P.K. & Bourne R.A. (1977). The organ culture of nodes of four species of *Eucalyptus*. Acta Hort. 78: 157-165.

de Fossard, R.A., Bennett, P.K., Gorst, J.R. & Bourne R.A. (1978). Tissue culture propagation of *Eucalyptus ficifolia*. F. Muell. Intern. Comb. Proc. Pl. Prop. Society. 28: 427-435.

De Little, D.W., Tibbits, W.N., Rasmussen, G.F. & Ravenwood, I.C. (1992). Genetic improvement strategy for APPM eucalypt tree farms in Tasmania. Mass production technology for genetically improved fast growing forest tree species, Bordeaux, AFOCEL.

Dell, B. & Malajczuk, N. (1989). Jarrah dieback - A disease caused by *Phytophthora cinnamomi.* In: The Jarrah Forest a Complex Mediterranean Ecosystem' In (eds.) Dell, B., Havel, J.J. & Malajczuk, N. (pp 67-87) Kluwer, Dordrecht.

Diallo, N. & Duhoux, E. (1984). Organogenése et multiplication *in vitro* chez *l'Eucalyptus camaldulensis*. J. Pl. Physiol. 115: 177-182.

Doi, K., Ho, K. & Shibata, M. (1987). Application of the shoot tip primordium method to the micropropagation of woody plants. Technical note (in Japanese, English summary). Oji Institute for Forest Tree Improvement.

Donald, D.G.M. & Newton, D.J. (1991). *In vitro* propagation of *Eucalyptus radiata.* Sth. Afr. For. J. 157: 66-68.

Dos Santos, V.P., D'Utra Vas & Power. J.B. (1990). Towards somatic hybridisation in the genus *Eucalyptus.* VII Intern. Congress on Plant Tissue and Cell Culture, Amsterdam. Abstract No A8-17: (p 208).

Durand-Creswell, R.J. & Nitsch, C. (1977). Factors influencing the regeneration of *Eucalyptus grandis* by organ culture. Acta Hort.78: 149-155.

Durand-Creswell, R., Bouley, M. & Franclet, A. (1982). Vegetative propagation of *Eucalyptus.* In Bonga, J.M. & Durzan, D.J. (eds) Tissue Culture in Forestry (pp 150-181). Martinus Nijhoff, The Hague.

Eldridge, K., Davidson, J., Harwood, C. & van Wyk, G. (1993). Eucalypt Domestication and Breeding. Clarendon Press, Oxford.

Evans, J. (1980). Prospects for eucalypts as forest trees in Great Britain. Forestry 53(2): 129-143.

Franclet, A. (1956). Premiers travaux d'amélioration génétique des *Eucalyptus.* Annales de Recherches Forestieres Rabat, Maroc 1: 65-89.

Franclet, A. & Boulay, M. (1982). Micropropagation of frost resistant eucalypt clones. Aust. For. Res. 13: 83-89.

Furuya, T., Orihara, Y. & Hayashi, C. (1987). Triterpenoids from *Eucalyptus perriniana* cultured cells. Phytochem. 26:715-719.

Furuya, T., Orihara, Y. & Miyatake, H. (1989). Biotransformation of (-)-menthol by *Eucalyptus perriniana* cultured cells. J. Chem. Soc. Perkin Trans 1: 1711-1719.

Furze, M.J. & Cresswell, C.F. (1985). Micropropagation of *Eucalyptus nitens* and *grandis* using tissue culture techniques. S. Afr. For. J. 135: 20-23.

Galli, M.A., Krugner, T.L., Goncalves, A.N. & Vieira, J.D. (1992). *In vitro* mycorrhizal synthesis in *Eucalyptus* plantlets inoculated with *Pisolithus tinctorius* in synthetic substrates. IUFRO/ AFOCEL Conference. Mass Production Technology for Genetically Improved Fast Growing Tree Species. Bordeaux France Sept. 1992 2: 227-235.

Gamborg, O.L., Miller, R.A. & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Goncalves, A.N. (1975). The growth and developmental physiology of *Eucalyptus* in cell and tissue culture systems. MSc Thesis. Ohio State University, Columbus (cited in Le Roux and van Staden 1991).

Goncalves, A.N. (1980). Revision of juvenility and cloning of *Eucalyptus urophylla* S.T. Blake in cell and tissue culture systems. IUFRO Symposium and Workshop on Genetic Improvement and Productivity of Fast Growing Tree Species. Sao Paulo, Brazil (pp 786-787).

Goncalves, A.N., Machado, M.A., Caldas, L.S., Sharp, W.R. & Mello, A.H. (1979). Tissue culture of *Eucalyptus.* In Sharp, W.R. (ed) Plant Cell and Tissue Culture: Principles and Applications. Ohio State University Press, Columbus (pp 509-526).

Gorst, J.R., Slaytor, M. & de Fossard R.A. (1983). The effect of indole-3-butryic acid riboflavin on the morphogenesis of adventitious roots of *Eucalyptus ficifolia* F. Muell. grown *in vitro.* J. Exp. Bot. 34: 1503-1515.

Grant, B.R. & Byrt, P.N. (1984). Root temperature effects on growth of *Phytophthora cinnamomi* in the roots of *Eucalyptus marginata* and *E. calophylla.* Phytophathology 74: 179-184.

Grattapaglia, D., Caldas, L.S., Machado, M.A. & Assis T.F. (1990). Large scale propagation of *Eucalyptus* species and hybrids. VII Intern. Congress on Plant Tissue and Cell Culture, Amsterdam. Abstract No A3-123, (pp 113).

Grewal, S., Ahija, A. & Atal, C. (1980). Clonal multiplication of medicinal plants by tissue culture. *In vitro* proliferation of shoot apices of *Eucalyptus citriodora* Hook. Indian J. Exp. Biol. 18: 775-776.

Gupta, P.K., Mascarenhas, A.F. & Jagannathan, V. (1981). Tissue culture of forest trees - clonal propagation of *Eucalyptus citriodora* Hook by tissue culture. Pl. Sci. Lett. 20: 195-201.

Gupta, P.K., Timmis, R. & Mascarenhas, A.F. (1991). Field performance of micropropagated forestry species. *In vitro* Cell. Dev. Biol. 27: 159-164.

Hartney, V.J. (1982). Tissue culture of *Eucalyptus*. Comb. Proc. Intern. Plant Prop. Soc. 32 :98-109.

Hartney, V.J. & Barker, P.K. (1983). The vegetative propagation of eucalypts by tissue culture. Silviculture 8: 791-792.

Holden, P.G. & Paton, D.M. (1981). Sterilisation of field grown *Eucalyptus* for organ culture. J. Aust. Inst. Hort. 3: 5-7.

Houran, W., Zeping, J & Hong, Y. (1994). Australian trees grown in China. ACIAR Proceeding No. 48, Zhangzhou, Australian Centre for International Research, Canberra.

Ilahi, I. & Jamal, S. (1987). Mass propagation of *Eucalyptus tereticornis Smith.* Pakistan J. Bot. 19: 67-74.

Ishii, K. (1982). Isoenzyme polymorphism of peroxidase of *Eucalyptus* callus in relation to plant hormone contents. In Proc. 5th Intern. Congress Plant Tissue and Cell Culture. Fujiwara, A. (ed.) Tokyo Japan pp 215-216.

Ito, K., Doi, K., Tatemichi, Y. & Shibata, M. (1990). Plant regeneration form protoplasts of *Eucalyptus.* VII Intern. Congress on Plant Tissue and Cell Culture, Amsterdam. Abstract No A1-65 (p 19).

Jacquiot. C., (1964a). Application de la technique de culture des tissue végétaux a l'etude de quelques probléms de la physiologie de l'abre. Ann. Sci. For. 21: 310-473.

Jacquiot. C., (1964b). Structure of excised roots or of organs formed *de novo* from cambial tissue of trees grown in culture. Rev. Cyt. Biol. Veg. 27: 319-322.

Janick, J. (1986). Horticultural Science 4th ed. WH Freeman, New York.

Jayawickrama, K.J.S., Schlatter, V.J.E. & Escobar, R. (1993). Eucalypt plantation forestry in Chile. Aust. For. 56: 179-192.

Jones, N.B. & van Staden, J. (1994). Micropropagation and establishment of *Eucalyptus grandis* hybrids. S. Afr. J. Bot. 60: 122-126.

Kapoor, M.L., & Chauhan, J.M.S. (1992). *In vitro* propagation of mature *Eucalyptus* F1 hybrid (*E. torelliana* F.V. Muell. x *E. citriodora* Hook). Silvae Genetica 41: 305-307.

Kawazu, T., Doi, K., Ohta, T., Shinohara, Y., Ito, K. & Shibata M. (1990). Transformation of *Eucalyptus (Eucalyptus saligna)* using electroporation VII Intern. Congress on Plant Tissue and Cell Culture, Amsterdam. Abstract No A2-80 (pp 64).

Kerruish, C.M. & Rawlins, W.H.M., eds. (1991). The Young Eucalypt Report. Canberra, CSIRO.

Kirdmanee, C. Kitaya, Y. & Kazai, T. (1994). CO2 enrichment and vermiculite medium enhanced photoautotrophic growth of *Eucalyptus* plants *in vitro.* (Abstract) VIIth Intern. Congress Plant Tissue and Cell Culture. Firenze (pp 57).

Knop, W. (1865). Quantative Untersuchunger uber den Ernahrangs process de Pfanzen. Landwirtschaftlichen Versuchsstationen. 7: 39-107.

Kuzminsky, E. & Lubrano, L. (1991). Impeigo di cloni micropropagati di *Eucalyptus globulus* di *Eucalyptus dalrympleana* per studi sulla resistenza al freddo. Monti. E Boschi 5: 51-53.

Laine, E. & David, A. (1994). Regeneration of plants from leaf explants of micropropagated clonal *Eucalyptus grandis.* Pl. Cell Rep. 13: 473-476.

Lakshimi Sita, G. (1979). Morphogenesis and plant regeneration from cotyledonary cultures of *Eucalyptus.* Pl. Sci. Lett. 14: 63-65.

Lakshimi Sita, G. (1982). Tissue culture of *Eucalyptus*. In Rao A.N. (ed) Tissue Culture of Economically Important Plants (pp 180-184) Singapore COSTED/ANBS.

Lakshimi Sita, G. (1993). Micropropagation of *Eucalyptus*. In Ahuja, M.R. (ed) Micropropagation of Woody Plants. (pp 263-280) Kluwer Academic Press, The Netherlands.

Lakshimi Sita, G. & Rani, S.B. (1985). *In vitro* propagation of *Eucalyptus grandis* L. by tissue culture. Plant Cell Reports 4: 63-65.

Lakshimi Sita, G. & Vaidyanathan C.S. (1979). Rapid multiplication of *Eucalyptus* by multiple shoot production. Curr. Sci. 48: 350-352.

Lee, E.C.M. & de Fossard R.A. (1974). The effects of various auxins and cytokinins on the in *vitro* culture of stem and lignotuber tissue of *Eucalyptus bancroftii* Maiden. New Phytol. 73: 707-717.

Le Roux, J.J. & van Staden, J. (1991). Micropropagation and tissue culture of *Eucalyptus* - a review. Tree Physiol. 9: 435-477.

Lloyd, G. & McCown, B. (1980). Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb. Proc. Intern. Pl. Prop. Soc. 30: 421-427.

Lubrano, L. (1991). Micropropagation of *Eucalyptus grandis*. Acta Hort. 300: 89-94.

MacRae, S. & van Staden J. (1990). *In vitro* culture of *Eucalyptus grandis*. Effects of gelling agents on propagation. J. Pl. Physiol. 137: 249-251.

MacRae, S. & van Staden J. (1993). *Agrobacterium rhizogenes*-mediated transformation to improve rooting ability of eucalypts. Tree Physiol. 12: 411-418.

Malan, F.S. (1988). Genetic variation in some growth and wood properties among 18 full-sib families of South African grown *Eucalyptus grandis:* A preliminary investigation. S. Afr. For. J. 146: 38-43.

Mascarenhas, A.F., Hazara, S., Potdar, U., Kulkarni, D.K. & Gupta, P.K. (1982). Rapid clonal multiplication of mature forest trees through tissue culture. In: Fujiwara, A. (ed.) Plant Tissue Culture 1982. Proc. 5th Intern. Congress Plant Tissue and Cell Culture, Tokyo, Japan (pp 719-720).

Mazanec, Z. (1989). Resistance of *Eucalyptus marginata* to *Perthida glyphopa* (Lepidoptera: Incurvariidae). J. Aust. Entomol. Soc. 24: 209-221.

Mazanec, Z. (1985). Jarrah leafminer, an insect pest of jarrah. In: (eds.) Dell, B., Havel, J.J. & Malajczuk, N. The Jarrah Forest a Complex Mediterranean Ecosystem (pp 123-31) Kluwer, Dordrecht.

McComb, J.A. (1995). Clonal propagation of eucalypts. In Lindsey K (Ed). Handbook of Plant Tissue Culture Manual Supplement 4. (08: pp 1-24) Kluwer, Dordrecht .

McComb, J.A. & Bennett, I.J. (1982). Vegetative propagation of *Eucalyptus* using tissue culture and its applications to forest improvement in Western Australia. In Fujiwara, A. (ed.) Plant Tissue Culture 1982. International Association of Plant Tissue Culture: Japan (pp 340-362).

McComb, J.A. & Bennett, I.J. (1986). *Eucalyptus* (eucalypts). In: Bajaj Y.P.S. (ed) Biotechnology in Agriculture and Forestry, (pp 340-362) Springer Verlag, Berlin. Vol I.

McComb, J.A., Bennett, I.J., van der Moezel, P.G. & Bell, D.T. (1989). Biotechnology enhances utilization of Australian woody species for pulp, fuel and land rehabilitation. Aust. J. Biotech. 34: 297-301.

McComb, J.A. Bennett, I.J. Stukely, M. & Crane, C. (1990). Selection and propagation of jarrah for dieback resistance. A progress report. Comb. Proc. Intern. Pl. Prop. Soc. 40: 86-90.

McComb, J.A., Stukely, M., & Bennett, I. (1994). Future ecosystems - use of genetic resistance. J. Roy. Soc. W.A. 77: 179-180.

McCown, B.H. & Lloyd, G.B. (1982). A survey of the response of *Rhododendron* to *in vitro* culture. Pl. Cell Tissue and Organ Cult. 2: 77-85.

McCown, D.D. & McCown, B. H. (1987). North American hardwoods. In: Bonga, J.M. & Durzan D.J. (eds.) Cell and Tissue Culture in Forestry Vol 3. (pp 247-260) Martinus Nijhoff, Dordrecht.

Mehra Palta, A. (1982). Clonal propagation of *Eucalyptus* by tissue culture. Pl. Sci. Lett. 26: 1-11.

Molleda, J. (1984). *Eucalyptus* pulp production and trade. Paper 202: 30-34.

Monod, V., Poissonier, M., Dereuddre, J. & Paques, M. (1992). Successful cryopreservation of *Eucalyptus gunnii* shoot tips in liquid nitrogen. IUFRO/AFOCEL Conference Mass Production Technology for Genetically Improved Fast Growing Tree Species, Bordeaux, France, Sept. 1992. 1: 133-145.

Muralidharan, E.M., & Mascarenhas, A.F. (1987). *In vitro* plantlet formation by organogenesis in *Eucalyptus camaldulensis* and by somatic embryogenesis in *Eucalyptus citriodora.* Pl. Cell Rep. 6: 256-259.

Muralidharan, E.M., Gupta, P.K. & Mascarenhas, A.F. (1989). Plantet production through high frequency somatic embryogenesis in long term cultures of *E. citriodora.* Pl. Cell Rep. 8: 41-43.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Mullins, K., Llewellyn, D.J. & Hartney, V. J. (1991). Regeneration and genetic transformation in *Eucalyptus camaldulensis.* Proceedings of the Australian branch of the international Association of Plant Tissue Culture. Launceston, July 14-18, 1991.

Newman, J.L. & Clarke, J. (1926). The jarrah leafminer. Aust. For. 9: 95-99.

Nitsch, C. & Nitsch, J.P. (1967). The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. I. The production of vegetative buds. Planta, 72:355-370

Oka, S., Yeng, E.C. & Thorpe, A. (1982). Shoot formation in *Eucalyptus globulus* hypocotyl explants. N.Z. J. Forestry Sci. 13: 501-509.

Orihara, Y., Saiki, K. & Furuya, T. (1991). Biotransformation of steviol by cultured cells of *Eucalyptus perriniana* and *Coffea arabica.* Phytochem. 30: 3989-3992.

Orihara, Y., Furuya, T., Hashimoto, N., Deguchi, Y., Tokoro, K. & Kanisawa, T. (1992). Biotransformation of isoeugenol and eugenol by cultured cells of *Eucalyptus perriniana.* Phytochem. 31: 827-831.

Ossor, A. & Boxus, P. (1992). Des Agglomérats de domes méristématiques: une nouvelle alternative dans la multiplication clonale d' *Eucalyptus camaldulensis* IUFRO/AFOCEL Conference Mass Production Technology for Genetically Improved Fast Growing Tree Species, Bordeaux, France, Sept. 1992. 2: 203-211.

Paton, D.M. (1983). Vegetative propagation of adult *Eucalyptus.* Colloque International sur les *Eucalyptus* Resistant au Friod. IUFRO/AFOCEL Bordeaux Sept 1983.

Penfold, A. & Willis, J.L. (1961). The Eucalypts. Leonard Hill, London.

Podger, F.D. (1972) *Phytophthora cinnamomi* , a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathol. 62: 972-981.

Pryor, L.D. (1976). The Biology of Eucalypts. Arnold, London.

Qin, C.L. & Kirby, E.G. (1992). *In vitro* responses of cultured explants of *Eucalyptus* spp. IUFRO/ AFOCEL Conference. Mass Production Technology for Genetically Improved Fast Growing Tree Species, Bordeaux, France, Sept. 1992 2: 213-218.

Quoirin, M. & Le Poivre, P. (1977). Étude de milieux adaptés aux cultures *in vitro* de *Prunus.* Acta Hort. 78: 437-442.

Raghavan, P. (1986). Plantlet regeneration from callus of *Eucalyptus grandis* Hill ex Maiden. Curr. Sci. 55: 741-743.

Rao, K.S. (1988). *In vitro* meristem cloning of *Eucalyptus tereticornis* Sm. Pl. Cell Rep. 7: 546-549.

Rao, K.S. & Venkateswara (1985). Tissue culture of forest trees: clonal multiplication of *Eucalyptus grandis* L. Pl. Sci. 40: 51-55.

Rockel, B. A. (1977). A simple method of inoculating large woody roots *in situ* with *Phytophthora cinnamomi* Rands. Aust. For. Res. 7: 271-272.

Salinero, M.C. (1983). Advances in micropropagation of *Eucalyptus globulus* by *in vitro* nodal culture. Colloque International sur les *Eucalyptus* Resistants au Froid. IUFRO/AFOCEL Bordeaux, Sept. 1983.

Samejima, M., Yamaguchi, T., Fukuzumi, T. & Yoshimoto, T. (1992). Effects of phytohormones on accumulation of flavanols in callus cells of woody plants. In A. Fujiwara (ed.) Proc. VIth Intern. Congress Plant Tissue and Cell Culture Tokyo. (pp 353-354).

Savory, B.M. (1962). Boron deficiency in eucalypts in Northern Rhodesia. Empire Forestry Review 41: 118-126.

Shearer, B.L. & Tippett, J.T. (1989). Jarrah Dieback: The dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin No. 3. Department of Conservation and Land Management, Western Australia.

Stephens, M., Hansard, A. & Dean, M. (1993). Competitiveness of selected Australian plantation forests. ABARE Research Report 93.10, Canberra.

Stone, E.L. (1968). Microelement nutrition of forest trees: A review. Forest Fertilisaton: Theory and Practice. Alabama, Tennessee Valley Authority. 132-175.

Stukely, M.J. & Crane, C.E. (1994). Genetically based resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. Phytopathol. (in press).

Subbaiah, M.M. & Minocha, S.C. (1990). Shoot regeneration from stem and leaf callus of *Eucalyptus tereticornis*. Pl. Cell Rep. 9: 370-373.

Sussex, I.M. (1965). The origin and morphogenesis of *Eucalyptus* cell populations. In White, P.R. & Groves, A. R. (eds) Proc. Intern. Conf. on Plant Tissue Culture (pp 383-391) McCutchan, Berkeley.

Teulieres, C. & Boudet, A.M. (1991). Isolation of protoplasts from different *Eucalyptus* species and preliminary studies on regeneration. Pl. Cell Tissue and Organ Cult. 25: 133-140.

Teulieres, C., Alibert, G., Marien, J.N. & Boudet, A. (1989a). Isolated and frost resistance screening of protoplasts from different clones of *Eucalyptus*. J. Pl. Physiol. 134: 316-319.

Teulieres, C. Feuillet, C. & Boudet, A.M. (1989b). Differential characteristics of cell suspension cultures initiated from *Eucalyptus gunnii* clones differing by their frost tolerance. Pl. Cell Rep. 8: 407-410.

Teulieres, C., Grima-Pettenati, J., Curie, J., Teisse, J. & Boudet, A.M. (1991). Transient foreign gene expression in polyethylene/glycol treated or electropulsated *Eucalyptus gunnii* protoplasts Pl. Cell Tissue and Org. Cult. 25: 125-132.

Teulieres, C., Rocharge, F., Serrano, L. Marque, C. & Boudet, A.M. (1994). DNA delivery into *Eucalyptus globulus* seedlings through biolistics (Abstract). VIIIth Inter. Congress Plant Tissue And Cell Culture, Firenze (pp 141).

Texier, F. & Faucher, M. (1986). Culture in *vitro* d'apex d'*Eucalyptus* agé. *Eucalyptus parvifolia* Comb. Annales de Recherches Sylvicoles 1985 AFOCEL Paris.

Thomson, L.A.S. (1981). Salt tolerance in eucalypts. Australian Salinity Newsletter No 9: 73.

Thomson, L.A.S. (1988). Salt tolerance in *Eucalyptus camaldulensis* and related species. PhD thesis Melbourne University.

Tibok, A., Davey, M.R. & Power, J.B. (1994). Plant regeneration from cultured hypocotyl explants of *Eucalyptus* and *Acacia* seedlings (Abstract) VIIIth Intern. Congress Plant Tissue And Cell Culture. Firenze. pp 165.

Tippett, J. T., Hill, T.C. & Shearer, B.L. (1985). Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust. J. Bot. 33:409-418.

Touchell, D.H. & Dixon, K.W. (1994). Cryopreservation of shoot tips of Australian rare and endangered plants. (Abstract) VIIIth Intern. Congress Plant Tissue And Cell Culture. Firenze. pp 20.

Tricoli, D. M., Maynard, C.A. & Drew, A.P. (1985). Tissue culture propagation of mature trees of *Prunus seritona* Ehrh. I. Establishment, multiplication and rooting *in vitro*. For. Sci. 31: 201-208.

Trindade, H., Ferriera, J.G., Pais, M.S. & Aloni, R. (1990). The role of cytokinin and auxin in rapid multiplication of shoots of *Eucalyptus globulus* grown *in vitro*. Aust. For. 53: 221-223.

Tripathi, B.K., Boulay, M. & Ben Badis, A. (1983). On the isolated root culture and root bud formation in *Eucalyptus viminalis* x *dalrympleana*. Colloque Intern. sur les *Eucalyptus* Résistants au Froid. IUFRO/AFOCEL Bordeaux Sept. 1983. (pp 602-608).

Umboh, I., Setianwan, I. Kanil, H., Yani Syansul & Situmorang, J. (1989). L'application de techniques de culture *in vitro* à la multiplications d' espèces forestières tropicales en Indonésie. Bull. Soc. Bot. Fr. 136: 179-184.

Ushiyama, M. & Furuya, T. (1989). Biotransformation of (RS) - tropic acid in suspension cultures of *Coffea arabica, Datura innoxia, Eucalyptus perriniana* and *Nicotiana tabacum*. Phytochem. 28: 2333-2339.

Van der Moezel, P.G. & Bell, D.T. (1987). Comparative seedling salt tolerance of several *Eucalyptus* and *Melaleuca* species from Western Australia. Aust. For. Res. 17: 151-158.

Van der Moezel, P.G., Watson, L.E., Pearce-Pinto, G.V.N. & Bell, D.T. (1988). The response of six *Eucalyptus* species and *Casuarina obesa* to the combined effect of salinity and waterlogging. Aust. J. Pl. Physiol. 15: 465-474.

Van der Moezel, P.G., Bell, D.T., Bennett, I.J., Strawbridge, M & McComb, J.A. (1992). The development of salt tolerant clonal trees in Australia. Comb. Proc. Intern. Pl. Prop. Soc. 40: 73-78.

Van Wyk, G. (1990). Genetic improvement of timber yield and wood quality in *Eucalyptus grandis* (Hill) Maiden. Part I. Genetic parameters of growth characteristics. Sth. Afr. For. J. 153: 1-11.

Venkateswara, R., Sankara Rao, S. & Vaidyanathan, C.S. (1986). Phytochemical constituents of cultured cells of *Eucalyptus tereticornis* Sm. Pl. Cell Rep. 3:231-233.

Wallace, M.M.H. (1970). The biology of the jarrah leafminer. *Perthida glyphopa* Common (Lepidoptera: Incurvariidae). Aust. J. Zool. 18: 91-104.

Wallington, B. (1992). Variation in Invertebrate Distribution Between Clonal Eucalypts. Hons. Thesis Murdoch University.

Warrag, E., Lesney, M.S. & Rockwood, D.J. (1991). Nodule culture and regeneration of *Eucalyptus grandis* hybrids. Pl. Cell Rep. 9: 586-589.

Watt, M.P., Blackeway, F., Cresswell, C.F. & Herman, B. (1991). Somatic embryogenesis in *Eucalyptus grandis*. S. Afr. For. J. 157: 59-65.

Weste, G. & Marks, G.C. (1987). The biology of *Phytophthora cinnamomi* in Australian forests. Ann. Rev. Phytophath. 25: 207-29.

Willyams, D., Mooljhuzen, P., Volkes, P., Raymond, C. & Chandler, S. (1992a). Micropropagation of juvenile *Eucalyptus regnans*. IUFRO/AFOCEL Conference Mass Production Technology for Genetically Improved Fast Growing Tree Species, Bordeaux, France, Sept. 1992. 1: 415-419.

Willyams, D., Whiteman, P., Cameron, J. & Chandler, S. (1992b). Inter- and intra- family variability for rooting capacity in micropropagated *Eucalyptus globulus* and *Eucalyptus nitens*. IUFRO/ AFOCEL Conference Mass Production Technology for Genetically Improved Fast Growing Tree Species, Bordeaux, France, Sept. 1992. 2: 178-181.

Yamaguchi, T. & Fukuzumi, T. (1982). Volatile compounds from callus cells of woody plants. In Fujiwara, A. (ed) Proc. Vth Intern. Congress. Plant Tissue and Cell Culture, Tokyo (pp 287-288).

Year Book Australia (1984) Number 69. Cameron, R.J. (ed). Australian Bureau of Statistics, Canberra.
Year Book Australia (1994) Number 76. Castles, I. (ed). Australian Bureau of Statistics, Canberra.
Zacharin, R.F. (1978). Emigrant Eucalypts - Gum Trees as Exotics. Melbourne University Press.

List of Species and Authorities

Eucalyptus botryoides Smith
E. calycogona Turez.
E. camaldulensis Dehnh.
E. cinerea F. Muell. ex Benth.
E. citriodora Hook.
E. comitae-vallis Maiden
E. dalrympleana Maiden
E. deglupta Blume
E. delegatensis R. Baker
E. dunnii Maiden
E. exserta F. Muell.
E. ficifolia F. Muell.
E. globulus Labill.
E. grandis W. Hill ex Maiden
E. graniticola (ms.)
E. gunnii J.D. Hook.
E. halophila D. Carr & S. Carr
E. kondininensis Maiden & Blakely
E. kumarlensis Brooker
E. lesoufii Maiden
E. marginata Donn ex Sm.
E. myriadena Brooker
E. nitens (Dean & Maiden) Maiden
E. nova-anglica Dean & Maiden
E. occidentalis Endl.
E. ovata Labill.
E. paniculata Smith
E. parvifolia Cambage
E. pauciflora Seiber ex Sprengel
E. perrineana F. Muell. ex Rodway
E. platycorys Maiden & Blakely
E. radiata Sieber ex DC.
E. regnans F. Muell.
E. robusta Smith
E. rudis Endl.
E. salicola Brooker
E. saligna Smith
E. sargentii Maiden
E. sideroxylon Cunn. ex Woolls
E. spathulata Hook.
E. tereticornis Smith
E. urophylla S.T. Blake
E. viminalis Labill.
E. wandoo Blakely
E. yilgarnensis (Maiden) Brooker
Hibiscus cannabinus L.
Perthida glyphopa Common
Phytophthora cinnamomi Rands
Pinus radiata D. Don
Pisolithus tinctorius (pers.) Cok & Couch