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Potential for Microprogation of the Hybrid Eucalypt grandis and Eucalyptus nitens

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POTENTIAL FOR THE MICROPROPAGATION OF THE HYBRID *EUCALYPTUS GRANDIS X EUCALYPTUS NITENS*

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INTRODUCTION

Inter-specific eucalypt hybrids are becoming increasingly important to commercial forestry operations. Presently hybrids of the subtropical eucalypts are most common and these include *Eucalyptus grandis* crossed with *E. cameldulensis, E. urophylla or E. tereticornis*. Hybrids have facilitated the planting of areas previously considered off-site for plantation forestry. On these marginal sites, growth and survival of the hybrids has outperformed the pure species, and they are consistently more resistant to diseases, pests, cold, heat and drought. Through developing hybrid combinations, land closer to processing sites can now be planted (Denison and Kietzka, 1993a). These desirable characteristics of hybrids may also be applied to *E. grandis* x *E. nitens,* a cold-temperature tolerant eucalypt.

Hybrids, used commercially, must be carefully screened over a range of growing sites to ensure that the selected clones are well adapted for site and product (Denison and Kietzka, 1993 a). Many of the *E. grandis* hybrids express heterosis (hybrid vigor) early in life but do not maintain the good growth through to rotation (sprinters). Denison and Kietzka (1993a) recommend that decisions of the acceptance of hybrid combinations for operational programmes should not be made until at least half-rotation age for the eucalypt hybrids (3-4 years in NZ). Provided careful screening and care is taken in selecting hybrid clones, meaningful yield gains, often in excess of 60%, are obtained when comparing clones to non-hybridised *E. grandis*.

McConnochie and Shelbourne (1996), Shelbourne *et al.* (1999) and Aimers-Halliday *et al.* (1999) have reported on the performance of a small trial of *E. grandis x E. nitens* hybrids in New Zealand. Early results indicated some improvement over the performance of controls of either species at selected sites. However, the authors felt that results would be greatly improved if a larger number of seedlings was available to choose from each family and that selection should be made at age three or four years for ability to propagate by cuttings or tissue culture (Shelbourne *et. al.,* 1999; Aimers-Halliday *et. al.*, 1999). These observations are supported by the South African experience, where of a 1,000 hybrid seedlings chosen for continuing trials, they expected only 1-2% to make it to commercial production (Denison and Kietzka, 1993a).

The hybrid *E. grandis x E. nitens* must be vegetatively propagated to retain integrity. Seedlings of the hybrids show a high incidence of abnormalities and field trials have indicated that progeny performance is variable. Individuals that perform well in the field must be chosen and propagated, propagation methods must be cost-effective and produce numbers of plants useful for commercial forestry. Micropropagation techniques have the potential to facilitate this process and are being used by a number of companies around the world.

Interest in a micropropagation system for *Eucalyptus* species was fuelled largely by reports indicating that large numbers of plants are able to be produced. The multiplication rates depend on species, clone, explant source and juvenility (Le Roux and Van Staden, 1991). Hartney

(1982a) achieved rapid multiplication rates for 12 eucalypt species, with a potential production of 100 million shoots from a single shoot within a year. *Eucalyptus marginata* seedling clones showed even higher multiplication rates (Bennet and McComb, 1982). Furze and Cresswell (1985) reported, based on experimental results, that it would be possible to produce 700,000 and 500,000 shoots of *E. grandis* and *E. nitens* respectively within a 12 month period. Warrag *et. al.* (1990), working with sub-tropical *E. grandis* hybrids, estimated that using their reported techniques and allowing for adequate elongation, rooting, and glasshouse survival, that they could produce 1 million plants from a single sterile explant. However, it appears that although these numbers have proved possible, it is too expensive to implement in a commercial operation.

Recent evidence also points to improved rooting from microcuttings taken from plants produced by tissue culture. This holds great promise for difficult-to-root clones, as is the case for *E. grandis x E. nitens* hybrids which have been bulked up through tissue culture, and are being used experimentally as parent material for microcutting production.

Micropropagation, as well as facilitating the amplification of stock plants and improving rootability of cuttings necessary for the application of clonal forestry, may also help with the import of desirable, field-proven clones from such countries as South Africa, Australia and Brazil for testing in New Zealand conditions.

In vitro culture may also enable large-scale collections of clonal material to be kept in cool storage or liquid nitrogen where they would be protected from adverse environmental conditions such as disease, insect attack and fire.

Genetic engineering, in combination with a micropropagation system, may have extra application with hybrids where sterility prevents more traditional breeding approaches. The introduction of such characteristics as insect and herbicide resistance would further increase the commercial value of hybrid eucalypts (Machado *et al.,* 1997).

The main aim of this report is to advise on the potential of tissue culture to assist with the development of a clonal forestry program for the hybrid *E. grandis x E. nitens* in New Zealand. Information reported here has been gathered from literature contained in peer-reviewed scientific journals, conference proceedings and personal communications with ***Forest Research*** colleagues and staff from commercial companies in New Zealand and South Africa. There is a small amount of literature available on the propagation of this particular hybrid, but more on the subtropical *Eucalyptus* hybrids, *E. grandis x E. urophylla* or *E. camaldulensis* and *E. territicornis*. There are also a number of references to *E. grandis* and *E. nitens* which were considered due to their obvious relevance to this project.

IN VITRO CULTURE, EXPLANT ORIGIN AND STERILISATION TECHNIQUES

Aseptic cultures of *Eucalyptus* have been established from seeds, seedlings, shoots, flowers and lignotubers. Micropropagation, through axillary proliferation and adventitious shoot proliferation on nodal explants or both, has been successful (Le Roux and Van Staden, 1991).

Seeds are relatively easy to sterilise, in that the seed coat is relatively impermeable when dry and also non-viable seed (floaters) are easy to separate. Seeds of *E. grandis x E. urophylla* were sterilised with 9% (v/v) sodium hypochlorite solution plus two drops of Tween 20 for 10 minutes, followed by rinsing five times in sterile distilled water (Barrueto Cid *et al.*, 1999).

Cultures have also been successfully established from coppice material of *E. grandis x E. urophylla*, providing it is pre-treated with fungicide to reduce contaminant load (Jones and Van Staden, 1994). Nodal shoots of juvenile coppice material, which had been regularly sprayed with 0.2% Benlate, were collected in the summer months (South Africa, January - March) and given a further treatment in 0.2% Benlate as a pre-sterilisation procedure. Intact branches containing 2-3 nodal shoots were surface-sterilised in 0.2% $HgCl_2$ (containing a few drops of Tween 20) for 15 minutes. The material was then rinsed several times in sterile distilled water. The sterile 2-3 nodal shoot material was trimmed into nodal sections (30-35mm in length), each containing two leaves. The leaves were reduced to one third of their original size and 80% of the explants remained contamination-free (Jones and Van Staden, 1994). However, some earlier work by DeFossard *et al.* (1977) did note that sterilisation of mature field-grown material had proved difficult because of endogenous microbial contamination. Warrag *et al.* (1990) compared shoots from girdled *E. grandis* hybrids with shoots from mature branches of the same trees. They reported 100% contamination in the tissue from mature material and 40% from the girdle shoots. Holden and Paton (1981) were able to sterilise field-grown *E. grandis* shoots by 75 minutes immersion in saturated Ca $(OCl)_2$, followed by 4 hours of UV irradiation. They obtained little contamination and 50% of the explants grew (the others sterilised to death?). These may be important considerations in collection of material where girdling/felling for coppice production of the chosen hybrids is inappropriate due to the field growing conditions.

Furze and Cresswell (1985) reported a simplified procedure for sterilising nodal segments from two-month-old seedlings and coppice shoots of *E. grandis* and *E. nitens,* which involved using a 10% sodium hypochlorite solution for 10 minutes, followed by rinsing 3 times in sterile distilled water. However, they did not state the percentage contamination or whether the stock plants were pre-treated with fungicides.

Further work has been reported with regard to fungal contaminants in *E. grandis* (Watt *et al.*, 1996) and these authors cited these contaminants of *Eucalyptus spp.* as the single most important cause of losses during micropropagation. They tested a range of commercially available anti-fungal agents and found some showed pronounced phytotoxic effects. Propamocarb hydrochloride (Previcur N) had a broad range of anti-fungal activity and was recommended as a good agent to use for the eradication of fungal infection in eucalypt cultures.

The establishment of cultures following sterilisation has not been without difficulties. Obviously, some of the sterilisation procedures described above are severe and the result has been dormancy and senescence, often with associated production of phenolic compounds which cause media and tissue browning. Le Roux and Van Staden in a review article (1991) compiled a list of general precautions to reduce phenolics and ultimately improve success of culture establishment for eucalyptus cultures in general. These steps included: selection of actively-growing tissue; trying to minimise the damage to tissue by sterilisation procedures, for example, keeping stock plants 'clean' as Jones and Van Staden (1994) did with Benlate spraying; washing sections of source material in running water prior to sterilisation; soaking explants in sterile water and/or antioxidant solutions and including antioxidants such as ascorbic acid in the media. Charcoal can also be of some benefit, although this can inhibit the action of plant growth regulators in the media. A short period of culture in the dark (7-14 days) and frequent subculture in the initial months of establishment were also cited as beneficial.

MEDIA

The most common basal medium used for *E. grandis*, *E. nitens* and hybrids of *E. grandis* organogenic tissue culture is half strength Murishige and Skoog (1962) medium (MS) Unless otherwise stated, the media detailed below are modifications to the half-strength MS medium; these modification details are described as the authors reported them.

Initiation

Warrag *et al.* (1990) modified their half-strength MS medium in several ways: by replacing the amino acid glycine with 200 mg/L of glutamine; increasing the thiamine to 1 mg/L; decreasing agar concentration to 6.5 gm/L and adding 10% coconut water to the basal medium (in general, the addition of coconut milk is not a good idea due to the lack of consistency in the product and mode of beneficial effect). The authors used this medium for 'pre-initiation' and kept the cultures in the dark to reduce phenolics and screen for contamination. The three clones tested were *E. grandis x E. tereticornis* or *E. camaldulensis* (they were not absolutely sure and the paper contains details of the identification procedures). Axillary bud initiation was achieved on medium that included 0.05 mg/L 1-naphtaline acetic acid (NAA) and 0.5mg/L 6-benzylaminopurine (BAP).

Barruetto Cid *et al.* (1999) initiated callus on seedlings of the hybrid *E. grandis x E. urophylla,* germinated *in vitro,* using 0.44 mg/L 1-phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea (thiadiazeron) (TDZ). They then tried various plant growth regulator formulations finding that 1.1 mg/L N6-benzyladenine (BA) and 0.01 mg/L NAA gave the best results in terms of health and shoot number.

Jones and Van Staden (1994) gave a detailed description of media used for *E. grandis x E. urophylla* tissue culture. Their initiation medium contained BA at 0.1 mg/L, polyvinylpyrrolidone (PVP) at 1 g/L (water-soluble), 20 g/L of sucrose and gelrite at 2 g/L.

Multiplication

The *E. grandis* hybrids used by Warrag *et al.* (1990) multiplied well on auxin-free medium that included 0.6 mg/L BAP. The authors also noted that continual subculture on this medium improved rooting significantly.

Multiplication medium used by Jones and Van Staden (1994) contained 0.2 mg/L BA, 0.01 mg/L NAA, 0.1 g/L myo-inositol, 0.1 mg/L calcium panthoenate and 0.1 mg/L biotin. PVP, sucrose and gelrite were as in the initiation medium.

Elongation

Shoots of *E. grandis* and *E. nitens* were elongated on medium containing 0.1 mg/L BA, 0.01 mg/L gibberellic acid, 0.1 mg/L NAA and 15 g/L activated charcoal (Furze and Cresswell 1985).

Warrag *et al.* (1990) found that their shoots elongated best on media with high auxin (2.5 mg/L indole-3-butyric acid -IBA) and cytokinin (1-1.5 mg/L zeatin).

Jones and Van Staden (1994) did not find the medium they used for elongation beneficial and shoots elongated variably within culture vessels. However, they found benefits to both elongation

and rooting with the addition of charcoal to the medium and this is detailed in rooting medium below.

Barrueto Cid *et al.* (1999) elongated their shoots on 1 μM BA, 0.5 μM NAA and 2 μM gibberellic acid and then transferred them to a growth-regulator-free medium for elongation. Some of these shoots rooted spontaneously, but all benefited from the rooting treatment detailed below.

Root Initiation

One of the considerations with root initiation is the balance between plant growth regulators. Too much auxin results in overt callus growth and can reduce rooting success with too little auxin, root meristems may not be initiated. The associated problems with callus formation is that the callus leaves scarring which may affect phloem and zylem transport between roots and foliage, any callus may also provide a suitable nutrition supply for pathogens which subsequently affect the vigour of the plantlet.

Warrag *et al.* (1990) used an initial period of dark culture (5 days) when shoots were placed on their root induction medium, which was a ¼ strength MS medium plus 2 mg/L of IBA. It is not clear from the report if the sucrose concentration was also a ¼ of normal.

E. grandis and *E. nitens* were rooted on a medium containing 1mg/L IBA, 15 g/L sucrose, as opposed to 20 g/L in initiation medium and shoots were also subjected to an initial dark period (Furze and Cresswell, 1985).

Jones and Van Staden (1994) tested 0.1 - 2.0 mg/L IBA in their rooting medium for *E. grandis* x *E. urophylla* without any significant difference between concentrations. Their best rooting was obtained with omission of plant growth regulators and myo-inositol and addition of 10 g/L activated charcoal to their multiplication medium. A further 0.5 g/L of gelrite was also added to this medium (activated charcoal tends to 'soften' media).

Barrueto Cid *et al.* (1999) rooted elongated shoots following a 5-day treatment with 2.5 μM IBA and 30 days treatment on a plant growth regulator-free medium that contained 1 g/L charcoal. The authors also found further subculture, 30-60 days on this medium, beneficial for rooting. Barrueto Cid *et al.* (1999) methods for culture of seedling material are complicated in comparison to Jones and Van Staden (1994).They also have failed to cite (find?) this reference and said that they were the first to report regeneration of *E. grandis* x *E. urophylla*. They may have benefited from the reference in that the higher charcoal concentration used by Jones and Van Staden, 10 g/L as compared to 1 g/L, may have reduced the time taken for the elongation and rooting phase. Their explant material is not ideal for the objectives of this *Eucalyptus* Co-op project, but are important to consider as the callus phase they obtained on the TDZ medium for initiation is important for genetic engineering, since callus is an ideal target tissue.

PLANTS IN THE GLASSHOUSE AND THE FIELD

In general, *in vitro*-grown *Eucalyptus* are very easily water stressed. Even in routine transfer situations under aseptic conditions, micropropagules show signs of turgor loss in foliage and care must be taken to transfer shoots quickly to fresh medium.

Regenerated plants need to be hardened to the natural environment, This involves a series of steps whereby the humidity around the plant is gradually reduced (from close to 100% *in vitro*) and plant metabolism is altered from partial dependence to full independence from an external carbohydrate source (simply: from sucrose as a C-source to atmospheric CO_2) (Sutter, 1988; Roux and Van Staden, 1991). Detailed below are some of the techniques and results reported in the literature for the hybrid *Eucalyptus spp.*

Warrag *et al.* (1990) reported that rooted plants transferred to mist conditions in a greenhouse showed an average of 82% survival, with significant variation between three hybrid clones. The number of roots formed on plantlets varied, with 62% of them having an average of 15 or more roots per plant after 3 weeks. Interestingly, the hybrid clone that had significantly more roots showed the poorest overall survival. Survival of propagules transferred from the mist conditions to the greenhouse was 100% after two months.

Jones and Van Staden (1994) hardened off rooted plants under conditions of intermittent mist and they reported a significant difference between the three *E. grandis x E. urophylla* clones, with one giving 70% success and the other two clones less than 40%.

Barrueto Cid *et al.* (1999) achieved good results following an IBA rooting treatment and the placing of plants in intermittent mist conditions. However, the proceeding *in vitro* culture protocol was complicated, involving a number of subculture transfers after the initial IBA exposure. It was not a cost-effective procedure and the tissue originated from hybrid seed that had not been field tested.

Field Trials of Micropropagated Plants

Rockwood and Warrag (1994) reported on a field trial of *E. grandis* where they compared micropropagated, macropropagated and seed-derived propagules from three ortets. At 57 months, they found no differences in tree height, DBH, volume, or survival between plantlet lines and between rooted cuttings and plantlets, but seedlings were inferior to plantlets and cuttings. Vegetative propagules were more uniform at every age, with typically less than one-half the variability observed among seedlings. The authors felt that even though plantlets and cuttings may be more expensive to produce, they had numerous advantages for *E. grandis* plantation establishment in Florida. Watt *et al.* (1995) established a trial with micropropagated and macropropagated plants from three *E. grandis* hybrids (*E. grandis x E. urophylla, E. camaldulensis* or *E. tereticornis*), and monitored tree height, survival, mean annual increment, DBH and size uniformity for three years. They found the macro- and micropropagated plants to be extremely uniform, and the microcuttings were even more uniform than the macrocuttings. For six out of the seven hybrids tested, plantlets produced from tissue culture outperformed those produced from macrocuttings, for all parameters measured. The authors did include a seedling control of *E. grandis*, but this performed poorly, possibly due to the hybrid vigour of the tested clones.

Another important discovery was made with the field performance of microcuttings of *E. grandis x E. nitens* planted for cutting stool beds. Micropropagation of *E. grandis x E. nitens* was shown to enhance subsequent rooting, especially with difficult-to-root clones (Denison and Kietzka 1993b). Comparisons were made using parent hedge material from tissue culture compared with material from rooted cuttings. There was improved rooting from plants produced through tissue culture compared with plants vegetatively produced from rooted cuttings. This was found to be

of particular significance with the cold tolerant *E. grandis x E. nitens* hybrids (Mondi Forests, 1999).

GENETIC ENGINEERING, AIMS AND ACHIEVEMENTS

There are exciting possibilities for eucalypt breeding programmes with the production of transgenic plants by gene transfer technologies. These technologies may be of particular significance with hybrids where elite clones have already been chosen for particular traits. Moreover, the introduction of new characteristics, such as sterility, modification of lignin content, insect resistance and herbicide resistance may increase productivity to meet the high demand for quality forestry products and low production costs (Machado *et al.,* 1997). Both Shell and ForBio companies have publicised the planting of transformed eucalypt field trials this year, though this information has been more from press releases than from scientific fora.

In the last ten years, there have been a number of papers presented, mostly at conferences, on transformation of *Eucalyptus* species; some of these are briefly listed here and details of paper title, proceedings and books are provided in the reference section. Chriqui *et al.* (1992) reported *Agrobacterium*-mediated transformation of *E. globulus* and *E. gunnii* and there are several more general papers reporting progress in genetic engineering of eucalypts including genetic engineering for insect resistance in temperate plantation *Eucalyptus* (Teulieres *et al.,* 1994; Edwards *et al.,* 1995; Harcourt *et al.,* 1995). Japanese researchers have reported transformation of *E. saligna* using electroporation (Kawasu *et al.*, 1990) and Macrae and Van Staden (1993) have described *Agrobacterium rhizogenes*-mediated transformation to improve the rooting ability of eucalypts. Mullins *et al.* (1995) have achieved regeneration and transformation (in that order) of *E. camaldulensis.*

Several authors have reported progress towards developing *Agrobacterium*-mediated transformation of hybrid *E grandis x E. urophylla* (Esmeraldo *et al.,* 1997; Andrade and Brasileiro, 1997). Both papers reported using *in vitro*-germinated seedlings as source material and one group of researchers produced shoots from tumours which showed GUS activity. They felt their procedure could be further optimised if they had a selection agent in the plant medium. At this stage they were having to assay all tumour-origin shoots (Andrade and Brasileiro, 1997). Machado *et al.* (1997) reported some interesting research with adult and elite clones of an *E. grandis* hybrid (they do not say 'which' hybrid); earlier work had tended to be with seedlings and genotypes of non-proven origin. The authors achieved long-term expression (as opposed to transient, which usually disappears after a growth cycle) and used a selection medium. They also regenerated shoots, although one gathers from the paper that this was an area where they felt some optimisation was required.

A report from 'The Economist' in August 1999, claimed that "Royal Dutch Shell had genetically engineered eucalypts to produce a different type of lignin, the molecular glue that holds wood fibres together. The oil multinational hopes its improved trees, growing in test sites in Uruguay and Chile, will prove a boom to the pulp and paper industry. Removing lignin is one the messiest bits of pulp production." To further support this, one of the seminars given at the recent joint meeting of the International Wood Biotechnology Symposium and the IUFRO working party 2.04.06 Molecular Genetics of Trees held in Oxford, United Kingdom in July, was from Horticulture Research International and Shell Forestry. D. Blakesley and R. Kiernan said that Shell forestry is using genetic modification strategies for the improvement of elite clones of an *E. grandis* hybrid. They did not say that there were already plants in field trials but instead

described the development of a cryopreservation system to store multiple transgenic lines while field evaluation took place - a process that could take up to five years in the case of altered wood quality.

Also, in the same Economist article, was a claim that ForBio hoped to have 10 million of its genetically enhanced trees growing around the world within two years. It already has fast-growing *Eucalyptus* in Indonesian plantations and hopes to get approval to plant its first crop of salt-tolerant trees in Australia's Murray Darling Basin. Unfortunately, ForBio is now in receivership, and so it is not known what has happened to this project.

STORAGE

In vitro culture may also enable large-scale collections of clonal material to be kept in cool storage or liquid nitrogen where they would be protected from adverse environmental conditions such as, disease, insect attack, and fire. Shoot cultures have potential for long-term storage of selected lines. Hartney (1982b) stored viable shoot cultures of *E. camaldulensis* and *E. grandis* on a simple medium in a domestic refrigerator for over eight months. Franclet and Boulay (1982) stored shoot cultures of *E. gunnii* and *E. dalrympleana* in the cold for six months without affecting their appearance or subsequent growth. Poissonier *et al.* (1991) stored alginate-encapsulated shoot tips of *E. gunnii* in liquid nitrogen and more recently Blakesly and Kiernan (1999) reported progress on developing cryopreservation techniques for eucalypt hybrids.

COMMERCIAL FORESTRY

Through informal correspondence with Mondi Forests and Fletcher Challenge Forests, as well as consideration of recent annual reports, it is obvious that propagation of *Eucalyptus* hybrids is an important area of focus. Companies have clearly identified the advantages of clonal forestry in combination with particular hybrids.

Mondi Forests is presently screening in excess of 1,000 hybrid clones of which only 1-2% will be commercialised. Provided careful screening and care is taken in selecting hybrid clones, mean yield gains, often in excess of 60%, are obtained when comparing clones to orchard seed of *E. grandis.* Denison and Kietzka (1993a) from Mondi Forests recommend that decisions of the acceptance of hybrid combinations for operational programmes should not be made until at least half-rotation age for the eucalypt hybrids (3-4 years in NZ). In 1997, Wex and Denison reported that the hybrid *E. grandis x E. nitens* had consistently produced high yielding hybrids which had been propagated clonally via a macro-cutting system then in use for the subtropical hybrids. Thus allowing the development of clonal forestry on a commercial scale for the colder regions of South Africa. Mondi Forests, at this stage had 142,000 ha of land holdings planted in temperate and cold-tolerant eucalypt species. In a recent communication with Neville Denison, he said that Mondi Forests was currently producing between 3 and 4 million cuttings of *E. grandis x E. nitens* via a combination of micro- and macro-cuttings and that demand was in excess of 8 million. He also said that micropropagation was used for about 10% of stock and the remaining 90% for macro-cuttings. The cost of producing more stock by micropropagation had proved too expensive. Micro-cuttings are used to provide hedges (or 'stool-beds') at Mondi Forests from which clonal cuttings are taken. Further conversations with laboratory staff confirmed that for *E. grandis x E. nitens,* micropropagated plants were not put into plantations but used as hedge plants from which cuttings were taken. All tissue culture was done via axillary buds, and they did

not use seed in culture. Their selections were made from trials where assessment had been made of wood quality and disease resistance characteristics. They were not allowed to do transformation at present due to their FSC certification.

Recently, Mondi Forests Tree Improvement Research (1999) reported that the interest in pure cold-tolerant and temperate species had waned. *E. grandis x E. nitens* hybrids, which are superior in form and growth, have been selected, because of their superior pulp properties and yield. Due to difficulties of rooting cuttings of cold-tolerant, clonal propagation of the cold-tolerant hybrids and pure species has however not been successfully achieved as has occurred with the sub-tropical eucalypts.

Fletcher Challenge Forests has an active interest in the propagation of *Eucalyptus spp.*, as well as involvement in gene mapping and transformation projects. For the past three and a half years, they have supported the Genesis Research and Development Corporation with the development of a cDNA library for *E. grandis* (and *Pinus radiata*). They have propagated more than 3,000 clones of *Eucalyptus* (species/hybrid breakdown not detailed) which have been planted in field trials in Argentina, Uruguay and New Zealand (Fletcher Challenge Forests, Annual Review 1999). Their primary interest in hybrids is with the cold tolerant *E. grandis x E. nitens* or *E. saligna.* Stock plants for clonal testing are from seedlings and field explants with proven performance (some imported through tissue culture). They currently use cold storage and field clonal archives for holding material that is being field-tested.

I also have made contact with Westvaco's South American eucalypt interests and hope to have some current status information for presenting at the meeting.

SUMMARY

Interest in *E. grandis* hybrids is intense, the gains realised to date from breeding, selection programs have not been over-estimated, and the potential for further improvement via traditional breeding programs working in tandem with advances in biotechnology are real. Many improvement and selection programs for the *E. grandis x E. nitens* hybrid are in progress overseas. Clonal material has been harvested, and growth rate, form and wood quality characteristics are known. Tissue culture can facilitate the importation and initial bulk-up of these clones and Fletcher Challenge Forests is taking advantage of this strategy.

Tissue culture has been shown to improve the subsequent rooting of cuttings taken from stool plants that were produced from micropropagated coppice explants. Stool plants, made from the same coppice material but with no 'micropropagation phase', do not produce cuttings that root as easily.

Tissue culture cool storage, or possibly cryopreservation, provides a method of storing material, including transformed material, until it is field proven or as insurance where there is a risk of fire, disease, or pestilence to archived material. It is also a way of keeping elite material readily available for customers.

The current state of the micropropagation technology for the *E. grandis* x *E. nitens* hybrid does not allow this technique to be used as a way of producing plants for the field, despite the high multiplication rates that have been demonstrated with other eucalypt species. Therefore, a combination of propagation technologies is recommended; firstly using tissue culture techniques

to assist with initial bulking-up, to improve subsequent rooting from stools, to allow importing and exporting of proven genotypes and possibly as a 'bank' for the elite resource. A watching brief should be kept on progress in the areas of gene mapping and transformation, rather than trying to replicate this work at ***Forest Research***.

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