VEGETATIVE PROPAGATION OF *EUCALYPTUS* USING TISSUE CULTURE AND ITS APPLICATION TO FOREST IMPROVEMENT IN WESTERN AUSTRALIA

J. A. McComb and I.J. Bennett

Environmental and Life Sciences, Murdoch University, Murdoch, Western Australia 6153

INTRODUCTION

Conventional vegetative propagation from mature eucalypts is not possible for most species, but micropropagation has been successfully used for several species (1, 2). Jarrah (*Eucalyptus marginata* Sm.), is an important forest species limited to Western Australia. There are some 1,400,000 ha. in state forest reserves, but 280,000 ha. of these have already been affected by dieback and the increase in a year may be up to 16,000 ha. A root pathogen, *Phytophthora cinnamomi* Rands appears responsible for the disease (3). Another serious pest is the leaf miner (*Perthida glyphopa* Common), a moth whose larvae damage leaf tissue (4). Healthy trees are occasionally found in dieback sites, and trees apparently resistant to leaf miner occur in the forest. We wish to clone these trees and test them for genotypically controlled resistance.

METHODS AND RESULTS

Initiation of shoot cultures

The youngest shoots from the crowns of mature trees are sterilized (10 min, 1% zephiran (benzalkonium chloride) in 10% alcohol). Contaminant free explants are difficult to obtain without also killing the plant tissue but ability to survive rigorous sterilization differs with the type of tree. Those resistant to leaf miner are simple to disinfect (Table 1). Nodal sections 1 cm long, preferably with an axillary bud 3-5mm long, are cut and placed in media.

Seeds can be surface sterilized, the seed coats removed, germinated under sterile conditions and their shoots excised for culture.

Table 1 Disinfestation of shoots from mature trees

Selection criteria	No. of trees attempted	Successful cultures
Fast growing trees	10	2
Dieback 'resistant' trees	35	5
Leaf miner 'resistant' trees	5	5

Shoot multiplication

The shoot multiplication medium is Murashige and Skoog (M & S; 5) minerals, vitamins and inositol (no glycine or casein hydrolysate), 60mM sucrose, 10g.l^{-1} agar, BAP 2.5µM and NAA 2.5µM. Shoots from mature trees show an average multiplication rate of x3 in four weeks, but the rate is lowest when cultures are first initiated; some do not start to multiply until after 6-10 months in culture. Clones of shoots from seedlings multiply immediately in culture at an average rate of x10.

Rooting

Rooting was initially found to be optimal in M & S major and minor minerals, B5 vitamins and inositol (6), agar 8g.l^{-1}, 60mM sucrose and 10µM IBA (7). Subsequently, reducing the level of the major minerals to ¼ strength (except for $CaCl_2$ which is optimal at ½ strength) has been found to slightly raise the percentage of rooting and to improve the vigour of the plantlets. Shoot cultures from mature trees show an initial low percentage rooting but this may rise 12 months or so after culture initiation (Fig.1). Similar changes in rooting frequency have been noted for other woody species in culture, including *Eucalyptus citriodora* (2). Shoot cultures from seedlings root at 5-80% with the higher frequencies most common. Plantlets show 50% survival on transfer

to soil.

Callus cultures

The difficulty of obtaining sterile material from adult trees and the long period elapsing before a high rooting percentage could be obtained, made us turn to callus cultures, though we are aware of the dangers of somaclonal variation. Previous reports of shoot regeneration from callus of mature trees are limited to lignotuber callus (8) though seedling callus is easier to regenerate (9, 10).

Flower buds are sterilized (5 min. 95% alcohol, then flaming, then 20 min. in 2% sodium hypochlorite) and stamens are removed and placed on the medium. The medium contains M & S major and minor minerals, B5 vitamins and inositol (6), 90mM sucrose, 10µM kinetin and 10µM NAA. Cultures are placed in the dark and callus develops from the filaments. On transfer to the light, and to a medium with 0.5µM IAA and 10µM zeatin, shoots may regenerate from the callus. Thus far callus of 4 out of 23 trees tested have yielded shoots. Shoots could be rooted, but like those from vegetative buds from mature trees they gave an initially low percentage rooting which improved only after 12 months in culture (Fig. 1).

GENERAL

The techniques described for jarrah shoot cultures also apply for other forest species such as E. calophylla (marri), E. diversicolor (karri) and E. wandoo. In the case of wandoo the purpose of selection is to develop salt tolerant lines. The genotypic differences which occur between mature trees in their rates of shoot multiplication and rooting in culture, make it difficult to predict the rate at which clones can be built up from a particular tree in the forest. Clones can readily be obtained from seedling explants.

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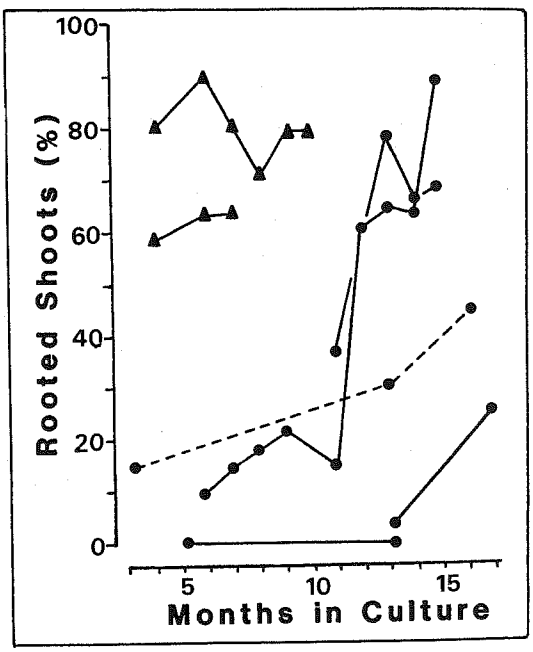

Fig. 1. Changes in rooting frequency of shoots at different times after culture initiation. ●—● Shoot cultures from four mature trees. ●- -● Shoots regenerated from stamen filament callus. ▲—▲ Shoot cultures from two seedlings.