GETTING STARTED IN MICROPROPAGATION OF TASMANIAN BLACKWOOD (*ACACIA MELANOXYLON*)

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Abstract. *Acacia melanoxylon* R.Br. plants have been regenerated from dissected embryos using tissue culture techniques. Shoots excised from seedlings grown *in-vitro* formed roots in a non-sterile environment following an *in-vitro* auxin/cytokinin treatment. The Quoirin-LePoivre (Q-LP) medium currently used for *in-vitro* culture is not optimal; addition of activated charcoal resulted in clones with less foliage abscission, larger shoots, more leaves, and higher leaflet numbers.

INTRODUCTION

Interest has been shown in Tasmanian blackwood (*A. melanoxylon*) as a plantation species as it grows reasonably fast and has an attractive, fine-grained timber suitable for furniture (1). Selection of clones with good stem form has not yet been undertaken in New Zealand, but clonal selection has been carried out in South Africa. Tissue culture techniques may provide early amplification of limited imported explant material.

Cutting propagation is not always successful with older material as rooting is often difficult. Micropropagation has worked with many species where cuttings have been unsuccessful and, although relatively expensive, can provide stock plants for further multiplication. With our present regulations, importation of cuttings and rooted stock involves lengthy delays for quarantine, because of the risk posed by insects and diseases carried on the foliage or in the roots and surrounding soil. Tissue culture may provide a better means of importing selected clones of *A. melanoxylon*. Disinfested micro-cuttings would guarantee insect-free material and greatly reduce the risk of introducing viral, bacterial, or fungal pathogens. On arrival in New Zealand, *in-vitro* material could be given rooting treatments and grown in a glasshouse environment to ensure that any stock released was free of pathogens, and also to aid the hardening-off of plant material. It could then be multiplied using conventional, less expensive, rooted cuttings.

Tissue culture of *Acacia* species has been reviewed by Skolman (2). Culture of *A. melanoxylon* is briefly described, and Bonner (2) is reported to have grown a root for more than a year in liquid culture. No plants were ever regenerated. Tissue culture of *Acacia koa* A. Gray is described more fully by Skolman (2), who reported that plants were regenerated from callus cultures derived from shoot tips. However the methods developed were very labour intensive and growth responses were slow.

Preliminary work with *A. melanoxylon* at the Forest Research

Institute, Rotorua, is described in this paper.

Seeds provide a ready source of sterile plant material. Plants grown *in-vitro* from these should provide useful leads for propagation of mature explant material. A potential application would be bulk vegetative "amplification" of limited amounts of seed from progeny-tested parent trees.

BASIC TECHNIQUES

Extraction of embryos from seeds. The seed used was from a parent stand at Jubilee Creek, South Africa. Seeds required scarification with a scalpel cut (Figure 1) before they could imbibe water and for the seed coat to become sufficiently soft to allow dissection of the embryos.

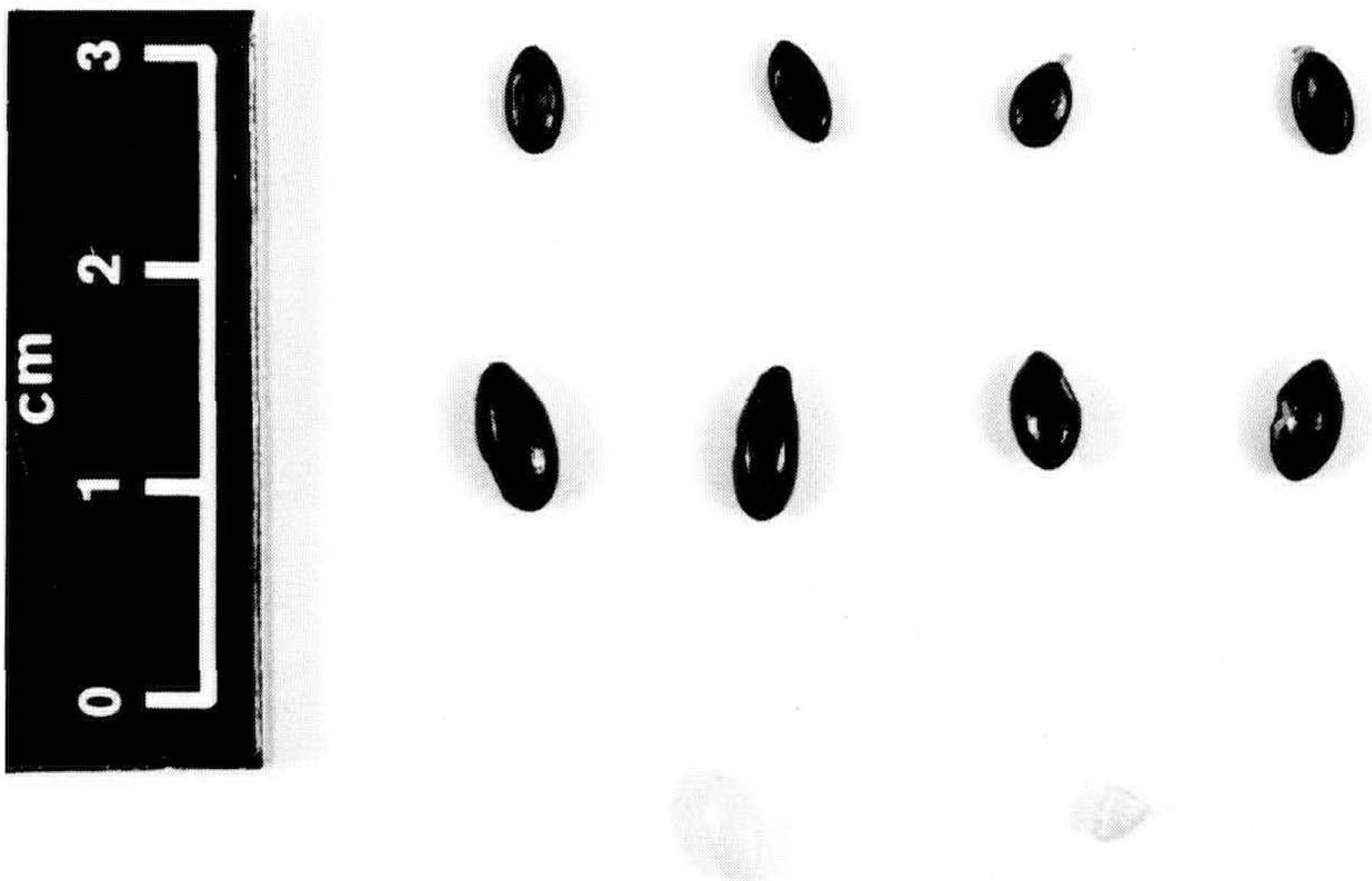

Figure 1. *Acacia melanoxylon* seeds. *Above*, pre-scarification. *Center*, after imbibition. *Below*, seed coats removed.

A number of surface sterilization methods were tested using a range of time exposures and sterilising agents. Initially it was hoped the seeds could be surface sterilised and germinated directly on the medium, but the seed coat provided a pool of contaminants; much less contamination occurred when the seed coat was removed. The best technique for dissecting out the embryos is detailed below:

1. Seeds were sterilised in a solution of 50% 'Chlorodux" containing a small amount of "Tween 80" (three drops per 100 mls) for 30 minutes. ("Chlorodux" is a commercial grade washing bleach and "Tween 80" is a concentrated detergent solution used as a surfactant).
2. The seeds were then rinsed in sterile water and, using

aseptic techniques, each seed coat was cut and the seed returned to sterile water for a further four hours imbibition.

3. The swollen seeds were soaked in a 5% hydrogen peroxide solution for 5 minutes and then rinsed in sterile water. (H_2O_2 hydrogen peroxide 100 vols concentration).
4. Seed coats were removed by hand (finger tips rinsed regularly in a 70% ethanol solution) and the top of the cotyledons removed.
5. Naked seeds were placed on an agar medium with radicle-end submerged.

In Vitro Growth. A 16-hour light/8-hour dark photoperiod was maintained throughout all stages of culture development; the "day" temperature varied between 21° and 25°C and the "night" temperature was 18° to 19°C.

Roots usually grew on the embryos and these were trimmed to approximately 10 mm in length to facilitate transfer to fresh media. Explants were transferred at 3- to 4-week intervals. Where sufficient elongation had taken place seedlings were topped and the excised shoot returned to the medium beside the original seedling.

Petri dishes (25 mm × 90 mm) were used for the first 7 to 10 days and all transfers after that were into 600-ml Agee jars containing approximately 100 mls of medium. Clear petri dish tops used for lids were held in place with thin plastic film (Gladwrap) wrapped around the jar rim.

FURTHER DEVELOPMENTS

Effect of Charcoal. A comparison was made of growth on Quoirin and Lepoivre (Q-LP) medium (3) with or without activated charcoal (Merck brand) at 2.5 gm/ litre. Shoots were rated for leaf abscission, and after 8 weeks in culture, shoot height was recorded. The numbers of compound leaves and leaflets were also recorded.

The incidence of foliar abscission was higher on the Q-LP medium without charcoal; this would have contributed to the overall lack of vigour observed on this medium (Table 1). Shoots on Q-LP+ charcoal had much larger, more normal-looking pinnae. The colour of the foliage ranged from yellow to green in both treatments.

Table 1. Summary of results after 8 weeks in culture (16 clones per treatment, 1 shoot per clone)

Medium	Clones with foliage abscission	Average height (cm)	Average leaf number	Average leaflet number
Q-LP	50%	1.3 ± 0.44	2.0 ± 1.86	5.4 ± 3.7
Q-LP + charcoal	12.5%	1.6 ± 0.40	4.0 ± 2.08	7.4 ± 4.1

Rooting Experiments. A root-initiation experiment was carried out using a small number of shoots that had been grown *in-vitro* for 4 weeks on charcoal medium after excision from seedlings. They were then grown in Q-LP medium containing 5 mg/l IBA (indolebutyric acid), 2.5 mg/l NAA (naphthaleneacetic acid), and 0.2 mg/l BAP (6-benzylaminopurine).

In all cases, shoots excised from the seedling *in-vitro* did not form roots at the base even after several months on hormone-free medium. Excised shoots placed in medium containing auxin and cytokinin were assessed after four weeks for adventitious root formation (Table 2). Some were put into potting mix, and some returned to a charcoal containing medium.

Table 2. Summary of results after four weeks on the Q-LP medium with auxin/cytokinin

Number of clones	Number of shoots	Shoots with callus	Shoots with roots in vitro
7	13	13	1

Of four callused shoots put out for rooting, two had produced roots (Figure 2). The shoot which formed roots *in-vitro* continued growth after potting up. The nine shoots left in culture did not form roots after 4 weeks although the callus became firm and nodulated.

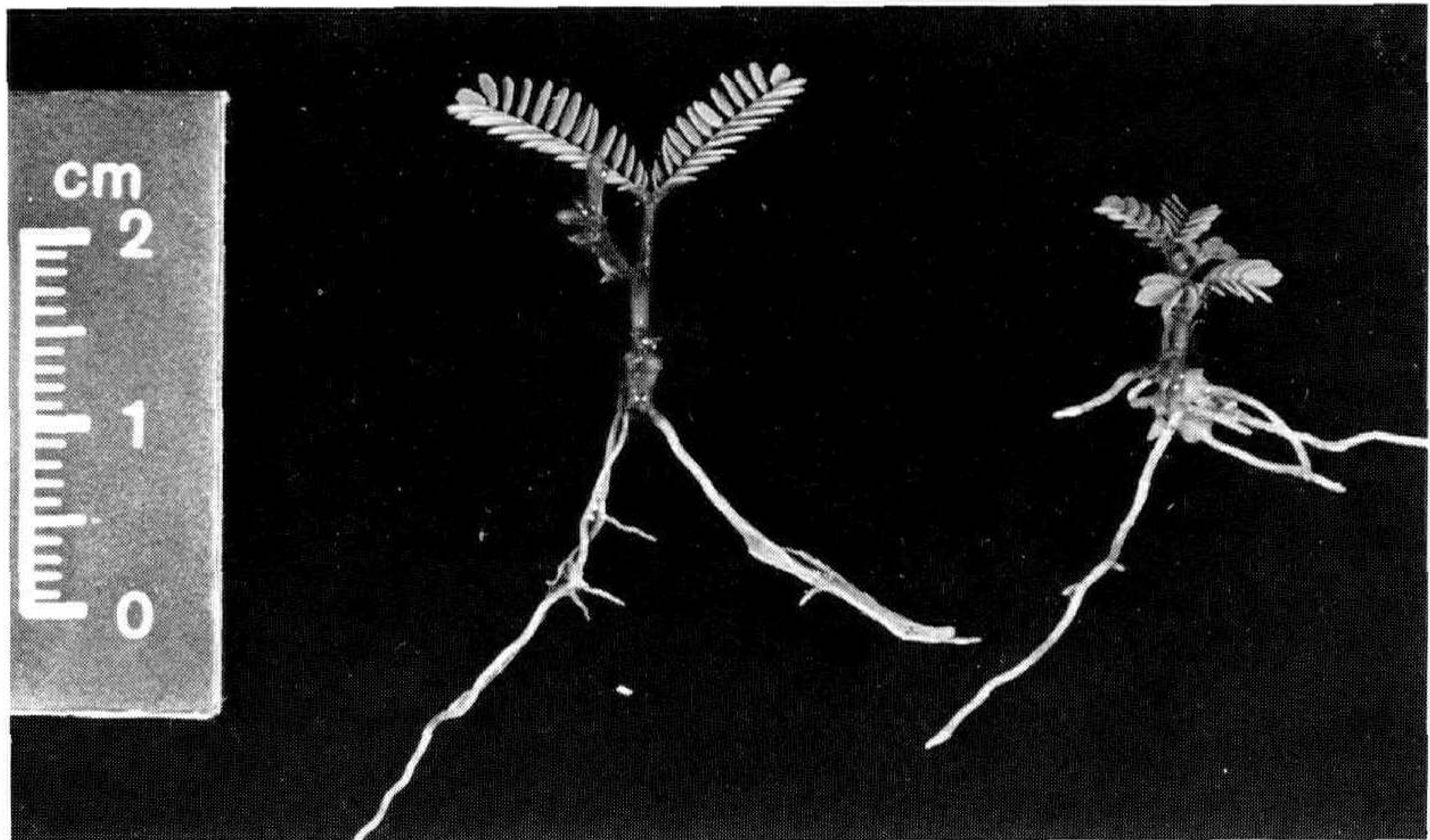

Figure 2. *Acacia melanoxylon* shoots that developed roots *in-vitro*.

CONCLUSIONS

This preliminary study shows that juvenile *Acacia melanoxylon* can be grown in sterile culture, and that shoots can be rooted in non-sterile conditions. Both the *in-vitro* and rooting stages

require further research to optimise media formulations. However, the feasibility of *in-vitro* multiplication of valuable seed from progeny-tested parents has been demonstrated. Further research will be necessary before the methods can be applied to field-grown *Acacia melanoxylon*.

LITERATURE CITED

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BIOLOGICAL CONTROL: DOES IT HAVE A PLACE IN PLANT PROPAGATION?

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Before the question posed in the title of this paper can be answered it is necessary to pose, and attempt to answer, two others:

(1) What is biological control? and (2) what can it achieve?

What is biological control? The term biological control can mean rather different things to different people. Like many well worked (or over-worked) terms it has been adapted and modified by various authors to suit their own particular view points. The way in which the term will be used in the present paper should be clear from the following discussion.

The basic ideas, concepts and early applications of biological control were developed primarily by entomologists who, at least 100 years ago, recognised the importance of natural enemies in regulating populations of pest species. To a large extent it is only during the past few decades that such concepts have been extended to organisms other than insects and mites. I will first discuss biological control with respect to pest insects, then consider briefly if and how it may be applied to other "pest organisms" in the broader sense.

A much quoted definition of biological control is that of DeBach (1), an entomologist: "*Biological Control* is the regulation