2) The micropropagation of endangered terrestrial orchids, in particular *Caladenia* spp., with the aim of producing plants for ex situ conservation collections and for reintroducing into natural habitats.

Research by Kirdmanee et al., (1995) using *Eucalyptus camaldulensis* suggests that the survival of plantlets ex vitro will be improved using this method.

Photoautotrophic micropropagation differs from standard micropropagation by maximising the potential of the explants/plantlets to photosynthesise and metabolise normally. It is an attempt to provide the conditions that allow for normal development of the plant. That is:

- Light is increased in the incubators using metal halide lights;
- Carbon dioxide in the atmosphere is increased so that it is not limiting for photosynthesis;
- Sucrose, which is implicated in the inhibition of the RubPcase enzyme, is not incorporated into the medium.

Horticultural research is now an important part of the Royal Botanic Gardens research program.

LITERATURE CITED

Kirdmanee, C., Y. Kitaya, and **T. Kozai.** 1995. Effects of CO_2 enrichment and supporting material in vitro on photoautotrophic growth of *Eucalyptus* plantlets in vitro and ex vitro: anatomical comparisons. Acta Hort. 393.

Micropropagation of *Evolvulus pilosus*

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INTRODUCTION

In Japan *Evolvulus pilosus* Nutt. 'Blue Daze' also known as "American Blue" has been popular as a potted ornamental for several years. Although the plant is easily propagated by softwood cuttings, micropropagation is expected to be the better technique for obtaining a large number of the elite clones of this plant. This paper describes the regeneration of the plant through organogenesis using three types of explants; nodal segments, shoot internodes, and leaf cuttings.

MATERIALS AND METHODS

Nodal segments (3 mm in length), shoot internodes (10 mm in length), and leaf cuttings were taken from a donor plant grown in a greenhouse. After sterilisation with 1% sodium hypochlorite solution, these explants were rinsed three times in sterile water and then placed on Murashige and Skoog (M&S) media supplemented with cytokinins. All media were adjusted to a pH between 5.7 and 5.8, and solidified with 0.2% Gelrite. In some experiments, nodal segments and shoot internodes taken from plantlets in vitro were used as explants. Shoots formed by the explants were transferred to a rooting medium supplemented with NAA. Cultures were kept at 25C with a 16-h photoperiod.

RESULTS

Axillary buds were rapidly induced from axillary meristem of the node explant. Induction of axillary buds in the range of 87% to 100% was achieved after 13 days in culture. Benzyladenine (BA) at 0.5 to 2.0 mg litre^{-1} had no effect on the induction of axillary buds. The rooting of axillary shoots was promoted by the addition of NAA at 0.05 mg litre^{-1} to the medium. Through this procedure genetically stable plantlets were ready for acclimatisation in approximately 75 days from the start of culture.

When the shoot internode explants were cultured on the media with BA added, callus and adventitious shoots were formed on the cut end of the explant. The formation of adventitious shoots was best on the medium supplemented with 1 mg litre^{-1} BA.

A large number of plantlets were produced using leaf cuttings as explants. Two types of shoot formation were observed. One was from the cut section of the leaf (by which it was divided into distal and proximal halves) and callus was often induced from the cut surface. The other was from the mid-vein near the petiole of the proximal half of the leaves. Benzyladenine had a greater positive effect on organogenesis of these explants than did isopentyladenine (2iP). Benzyladenine at 2 to 3 mg litre^{-1} resulted in the highest adventitious shoot formation per explant. The formation of adventitious shoots from the cut section of the distal half of the leaves was greater than that from the proximal half.

SUMMARY

Micropropagation can be used successfully to obtain a large number of clonal plantlets. Three types of explant material can be used; leaf cuttings on M&S medium supplemented with 3 mg litre^{-1} BA, shoot internodes on M&S medium supplemented with 1 mg litre^{-1} BA, and nodal segments on M&S medium with no added BA. Adventitious shoots or axillary buds will be induced depending upon the source of the explant material. All plantlets should be transferred to a rooting medium (M&S plus NAA at 0.05 mg litre^{-1}). Once roots have initiated the plantlets can be acclimatised and eventually moved to a greenhouse environment for growing on.

Propagation of *Michelia* and *Manglietia*

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SEED

Plants produce clusters of seed pods varying from a few to 20 or more capsules, each containing one or two seeds. Hard black seeds are surrounded by flesh varying in colour from orange to pink or red. Pick the capsules when they first begin to split or show colour when exposed by cutting. Split open fully to remove seed from capsules, squash the flesh or remove from around the seed. Some growers recommend washing the oil from the seed with detergent in case this inhibits germination. The only species for which we find this may be necessary is *Michelia champaca*, which has been difficult to germinate. Most varieties germinate easily if the seed is fresh. The seed should not be allowed to dry out as viability drops markedly. Under