A Technique for Field Collection of Woody Plants for Micropropagation©

Annemarie vd Westhuizen and Lorna Fischer
Shaw Research Centre, Sappi Forests, P.O. Box 473, Howick, 3290 South Africa
Email: annemarie.van.der.westhuizen@sappi.com

One of the biggest obstacles with the initiation of woody plants in tissue culture is contamination. Even when keeping the mother material in sheltered areas, pruning to encourage the growth of new material and treating regularly with broad-spectrum fungicides, it is not uncommon to observe contamination percentages greater than 90% during initiation.

Sterilization protocols for the majority of woody plants require the use of mercuric chloride ($HgCl_2$), but this it is not an environmentally friendly option. Calcium hypochlorite [$Ca(OCl)_2$] can be used as an alternative but is not as effective. Using a sterilization protocol adapted from other woody plants, which includes the use of both calcium hypochlorite and sodium hypochlorite (NaOCl) in an attempt to initiate *Eucalyptus* species into culture resulted in 100% contamination. After applying a technique on pre-sterilization storage for 48 h in the dark developed by Watt et al. (2003) explant contamination was reduced to 9%. Although the trials were conducted only on *Eucalyptus* it is a technique that has the potential for use in initiation of other woody species in tissue culture. The aim of this study was to assess the adaptability of a reported eucalypt-field-collection technique on the successful decontamination of eucalypt hybrid material.

INTRODUCTION

Eucalyptus micropropagation is used for the mass propagation of improved genetic material used in clonal programme activities in forestry. Tissue culture techniques can be used by the forestry industry for the propagation of selected genotypes in the breeding programmes, bulking up of hybrid genotypes, or replacement of nursery hedge stock (Watt et al., 2003). For effective in vitro multiplication a process of direct organogenesis from axillary buds was used as described in Jones and Van Staden (1997).

MATERIALS AND METHODS

Surface Sterilization for Micropropagation. For eucalypts hedge plants are normally used as stock material for micropropagation, because they can be kept in optimal growing conditions. In order to harvest the best quality material, the hedges must be exposed to optimal temperature, irrigation, and fertilization regimes, determined by the specific growing conditions of the clone. A strict fungicide regime must be employed to reduce the prevalence of endogenous pathogens causing the development of contamination after initiation in vitro (Watt et al., 2003).

After new developing shoots (coppice) are harvested from hedges, they are surfaced sterilized before being paced onto initiation medium. New shoots arising from the axillary buds are then harvested and transferred onto a multiplication medium. Surface sterilization techniques are not 100% effective, but a success rate of 5%–10% can be expected with woody plants. For the surface sterilization of eucalypts a

0.2 $g \cdot L^{-1}$ $HgCl_2$ solution is recommended (Watt et al., 2003). The use of $HgCl_2$ is hazardous, not environmentally friendly, and difficult to discard. For an alternative, 10 $g \cdot L^{-1}$ calcium hypochlorite (HTH) and a 40% dilution of commercial sodium hypochlorite supplemented with a few drops of Tween 20 can be used. Unfortunately this alternative is not always effective and a 100% fungal and bacterial contamination developed when *Eucalyptus* shoots were placed in tubes on initiation medium.

Collection and Storage Technique. A field collection technique was developed by Watt et al. (2003) where *E. grandis* sprouted shoots of approximately 100 mm with preformed apical and axillary buds were harvested, sprayed with 70% (v/v) alcohol, placed upright (5 per bottle) in autoclaved glass bottles containing 3–4 g sterile-water-moistened vermiculite and stored for 48 h at 24–26 °C. This technique was applied to eucalypt hybrid shoots harvested from hedges.

Culture Conditions. After the storage period, the shoots were surface sterilized with 10 $g \cdot L^{-1}$ calcium hypochlorite HTH for 2 min. and a 40% dilution of commercial sodium hypochlorite (Jik) with Tween 20 for 5 min. Sterile water rinses were conducted after use of each sterilant.

The explants were then placed, for approximately 10 days, on a bud initiation medium, containing MS nutrients, 20 $g \cdot L^{-1}$ sucrose, 4 $g \cdot L^{-1}$ Gelrite, and the pH was adjusted to 5.8. Developing buds were harvested and transferred for a further 4–5 weeks to a multiplication medium containing the same salts, sucrose and Gelrite concentrations as the initiation medium, but supplemented with 0.01 $mg \cdot L^{-1}$ NAA, and 0.2 $mg \cdot L^{-1}$ BA. The cultures were maintained under a 16-h light 8-h dark photoperiod at 24–26 °C.

RESULTS

The first time the pretreatment technique was applied, 17% contamination developed and the second time only 9%. It was also observed that leaving a leaf segment attached to the shoot did not influence the results. After the new buds were harvested, the shoots were placed on new initiation medium and further shoots developed from the same axillary buds, 10 days after transfer to similar light and temperature conditions. The percentage secondary bacterial infection that normally develops was also very low. Although this technique was developed for *Eucalyptus*, it is possible to apply it to other woody plants that also are also prone to endogenous bacterial and fungal contamination when in vitro regeneration from shoots is attempted.

LITERATURE CITED

Jones, N.B., and **J. Van Staden.** 1997. Micropropagation of *Eucalyptus*. Biotech. Agric. Forestry 39:286–329.

Watt, M.P., P. Berjak, A. Makhatini, and **F.C. Blakeway.** 2003. In vitro field collection techniques for *Eucalyptus* micropropagation. Plant Cell Tissue Organ Culture 75:233–240.