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In vitro field collection techniques for *Eucalyptus* micropropagation

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Abstract

A simple method was established for the collection and short-term storage of shoot material from field-grown *Eucalyptus grandis* and *E. grandis* hybrids for micropropagation. Initial studies were undertaken with plants grown outside a greenhouse, which were neither fertilised nor treated with fungicides. The method was then tested and adapted for field-grown clones. It involved collecting 35–50 mm long stems with three nodes and no leaves, spraying them with 70% (v/v) ethanol and storing them in glass bottles containing moist sterile vermiculite for 48 h. Addition of 1 g l^{-1} calcium hypochlorite to the first culture medium (bud break) inhibited endogenous contamination. Multiplication yields after storage of field-grown explants were 160–264 shoots/100 explants, depending on clone. This offers an alternative, improved means for explant collection over present standard procedures of maintaining parent plants in hedges or transporting shoots to the micropropagation laboratory in buckets.

Introduction

The ability to mass propagate improved genetic material produced through breeding and clonal programme activities is highly desirable. Large forestry and forest products companies use tissue culture systems for the mass production of selected genotypes for successive breeding generations, bulking up of hybrid genotypes, replacement of nursery hedge stocks and for commercial deployment of selected clones. The commonly used approach is to multiply mature selected genotypes (clones) *in vitro* through direct organogenesis from axillary buds, a technique for which specific protocols have been established for numerous *Eucalyptus* species and hybrids; this is routinely performed in many commercial forestry laboratories (le Roux and van Staden, 1991; Watt et al., 1999, 2000, 2003 (in press)).

The foundation of clonal programmes is the multiplication of highly selected (superior) genotypes which, in the case of tree species, are mature plants often grown in remote sites, at long distances from the laboratories and propagation nurseries. This results in excessive losses of explants in the initial stages of culture due to microbial contamination. Shoots collected in the field wilt and endogenous microbial contaminants proliferate during the usually long (up to 72 h in our experience) period between field collection and culture initiation in the laboratory. For these reasons, the common practices for the *in vitro* multiplication of selected *Eucalyptus* genotypes is to harvest coppice from field plants, set cuttings and then use the established plants in the greenhouse, or in hedges, as stock material for micropropagation. There is, therefore, a considerable delay (8–12 months) before explants for *in vitro* culture can be harvested from this greenhouse stock material. An alternative approach that would accelerate micropropagation is *in vitro* collection, i.e., surface sterilisation of material in the field and transport under semi-sterile conditions (Ashmore, 1997). Although this approach has proven successful for some species (e.g. coconut, Assy-Bah et al., 1987; cacao, Yidana et al., 1987), to our knowledge, it has not been reported for *Eucalyptus*.