The Use of *Meta*-Topolin as an Alternative Cytokinin in the Tissue Culture of *Eucalyptus* Species©

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***Meta*-topolin (*m*T) is a relatively new cytokinin isolated from poplar leaves in 1975 and is closely related to 6-benzyladenine (BA). Research on the use of *m*T in tissue culture has been conducted on several species, including *Hypericum*, citrus rootstock, *Aloe*, banana (*Musa acuminata*), pineapple (*Ananas comosus*), and *Barleria*. 6-Benzyladenine (BA) is the most widely used cytokinin in the regeneration stage of the tissue culture of most plant species, because of its availability and price, but it has a few drawbacks which include causing the hyperhydricity (vitrification) of shoots and it can have a negative effect on rooting. In light of this a series of trials were initiated to determine the effect of *m*T on the regeneration, hyperhydricity, and rooting of *Eucalyptus* species. In the initial trial various concentrations of *m*T (ranging from 1.2 to 14.5 mg·L^{-1}) were tested with the resulting shoot growth compact and stunted. In a follow-up trial, a *m*T concentration of 0.2 mg·L^{-1} was found to produce shoots that were less vitrified and that resulted in better in vitro rooting. Further trials on rooting of other eucalypt species are in progress to determine the benefits of *m*T.**

INTRODUCTION

In the in vitro propagation of plants a high cytokinin:auxin ratio is typically used to induce multiplication or regeneration (Murashige and Skoog, 1962), but the types and concentrations of auxins and cytokinins used vary between species (Niedz and Evens, 2010). The cytokinin most widely used is 6-benzyladenine (BA) or otherwise known as BAP (6-benzylaminopurine) because of its availability, effectiveness and affordability (Bairu et al., 2007). This cytokinin has a few drawbacks such as causing the hyperhydricity (vitrification) of plant shoots and can also have a negative effect on rooting in some species. In research conducted with *meta*-topolin (*m*T) these negative effects have not been evident (Meyer et al., 2009; Bairu et al., 2007). Alternative cytokinins include kinetin, zeatin, or thidiazuron (TDZ) and their use depends on the species propagated. The choice of cytokinin is determined by the cumulative efficiency in inducing an acceptable rate of multiplication, normal shoot and root development as well as ease of acclimatization. *Meta*-topolin is a relatively new cytokinin isolated from poplar leaves in 1975 and is closely related to BA (Strnad et al., 1997; Teklehaymanot et al., 2010). Meyer et al. (2009) found that *m*T is twice as effective as BA in the induction of shoot cuttings. Hyperhydricity usually increases with an increase in cytokinin concentration. When comparing BA with *m*T, no hyperhydricity developed in the shoots and higher multiplication rates were obtained. Plants also rooted spontaneously in the multiplication media (Bairu et al., 2007; Meyer et al., 2009). When *m*T was used in the multiplication of banana, superior multiplication rates were recorded (Bairu et al., 2008; Escalona et al., 2003). Research on the use of *m*T in tissue culture has been conducted on several species, for instance *Hypericum* (Meyer et al., 2009), citrus rootstock (Niedz and Evens, 2010), pineapple (Teklehaymanot et al., 2010), and *Barleria* (Amoo et al., 2011).

Research by Bairu et al. (2007) showed the effect of different cytokinins on the ex vitro growth of *Aloe polyphylla*. The addition of both 2.5 and 5.0 µM *m*T (0.6 and 1.2 mg·L^{-1}, respectively) to full strength Murashige and Skoog medium (MS) gave better growth and root formation than with BA and Zeatin.

MICROPROPAGATION OF EUCALYPTUS SPECIES

Benzylaminopurine is the standard cytokinin used for the propagation of several *Eucalyptus* species. When using a protocol developed by Jones and van Staden (1994) for

Eucalyptus grandis × *E. urophylla* (GU) with BA as the cytokinin, hyperhydricity developed in the shoots (Fig. 1a), which resulted in low survival and rooting (±40%). Since *m*T has been extensively studied at the University of KwaZulu-Natal, attempts were made to test this product to alleviate shoot vitrification. A trial was conducted to determine the effect of *m*T on the regeneration, hyperhydricity, and rooting of GU hybrids. The concentrations of *m*T tested in the initial trial were 1.2, 2.0, 6.0 and 14.5 $mg \cdot L^{-1}$ in full strength Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium, supplemented with 20 $g \cdot L^{-1}$ sucrose, 0.01 $mg \cdot L^{-1}$ α-naphthalene acetic acid (NAA), and 8 $g \cdot L^{-1}$ agar, with a pH of 5.8. Plants were kept in a 16-h light and 8-h dark photoperiod under cool white fluorescent lights. The resulting shoot growth was compact and stunted on all concentrations tested. In a follow-up study the concentrations of *m*T were significantly reduced and 0.2 $mg \cdot L^{-1}$ was determined as the optimum concentration for multiplication (Fig. 1b).

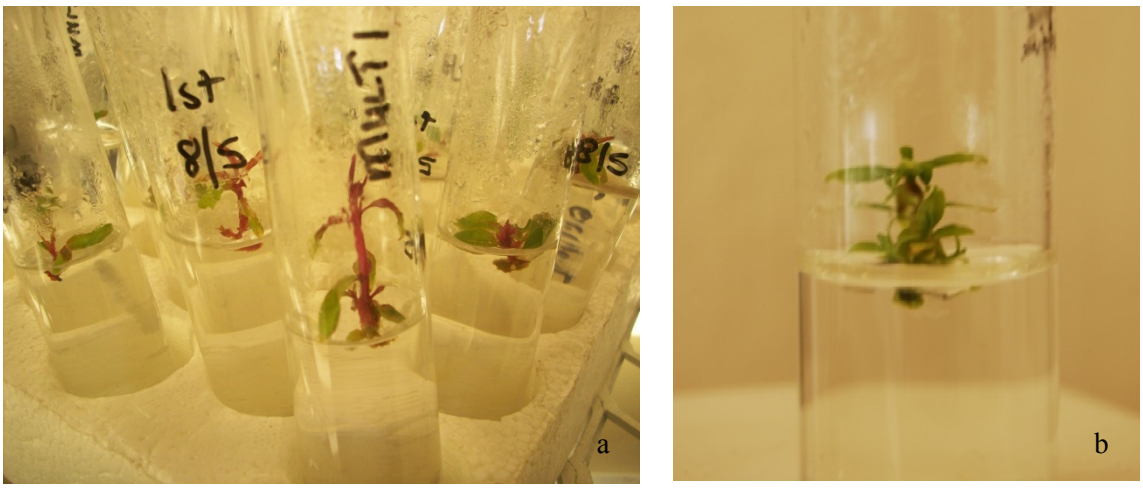

Fig. 1. Multiplication of *Eucalyptus grandis* × *Eucalyptus urophylla* hybrids on (a) MS medium supplemented with 2.0 $mg \cdot L^{-1}$ BA and (b) Murashige and Skoog medium supplemented with 0.2 $mg \cdot L^{-1}$ *meta*-topolin.

The shoots obtained from the second trial were transferred to rooting medium consisting of half strength MS salts, 0.2 $mg \cdot L^{-1}$ IBA (indole-3-butyric acid), 20 $g \cdot L^{-1}$ sucrose, 10 $g \cdot L^{-1}$ activated charcoal and 8 $g \cdot L^{-1}$ agar. A 20% increase in rooting was obtained (±60-80%) with the use of *m*T in the multiplication medium instead of BA (data not shown).

The rooted shoots also acclimatized very well with a 90% survival rate after it was planted in a pine-bark medium, closed with plastic. After the first week, the plastic was gradually removed in a greenhouse with high humidity (±75%) and bottom heating. As the plants acclimatized they were moved to a lower humidity.

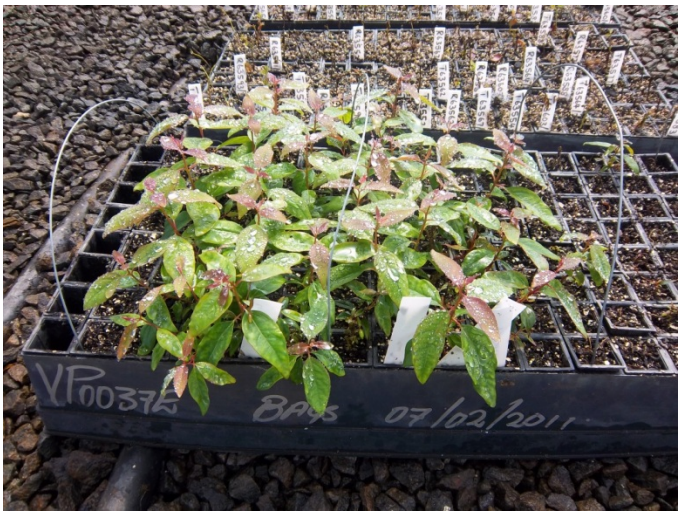

Fig. 2. Well-developed, acclimatized, rooted *Eucalyptus grandis* × *Eucalyptus urophylla* plants after *meta*-topolin was used as the cytokinin source during in vitro multiplication.

The same protocol was also applied to a temperate *Eucalyptus* species, *E. dunnii*, with similar results (Figs. 3). The higher *m*T concentration (1.2 mg·L^{-1}) caused compacted and stunted growth, while well-developed plants were obtained using the lower *m*T concentration of 0.2 mg·L^{-1} *m*T, with no apparent hyperhydricity (Fig. 3a). After 5 weeks in culture shoots grown on 0.2 mg·L^{-1} *m*T were well developed and normal in appearance (Fig. 3b). The rooting trials of with this species are not yet completed.

Fig. 3. Multiplication of a temperate *Eucalyptus* species using (a) 0.2 mg·L^{-1} *meta*-topolin (*m*T) (left) and 1.2 mg·L^{-1} *m*T (right), and (b) 0.2 mg·L^{-1} *m*T.

A major problem with the use of alternative cytokinins is their affordability. The prices and possible suppliers of various cytokinins are given in Table 1.

Table 1. Comparison of cytokinin prices as at April 2013.

Cytokinin	Supplier	Price/g (R)
Benzylaminopurine (BA)	Sigma-Aldrich	330
Kinetin	Sigma-Aldrich	925
Zeatin	Sigma-Aldrich	53,000
Thidiazuron (TDZ)	Sigma-Aldrich	43,300
meta-Topolin	Duchefa (Labretoria)	7,500

The most affordable cytokinin is BA, but for *Eucalyptus* propagation, use of *m*T at 0.2 $mg \cdot L^{-1}$ was more efficient than the use of BA at 2 $mg \cdot L^{-1}$ (Table 2). At this rate *m*T is only approximately double the price of BA and much less than that for zeatin and TDZ. Trials to test the efficacy of kinetin have not been conducted since several researchers have indicated that kinetin cannot be used continually for successful in vitro propagation of *Eucalyptus* species.

SUMMARY
With the production of plants through tissue culture, the quality of the shoots obtained is extremely important as this determines the ability of the shoots to be rooted and acclimatized effectively. Although *m*T is more expensive per gram than BA, the quality of the plants is better and consequently better rooting can be obtained. Further trials on rooting of the temperate *Eucalyptus* species are in process to determine the most effective protocol.

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