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A method to induce adventitious rooting in microshoot cultures of *Thryptomene ericaea* (Myrtaceae)

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Abstract

A protocol to induce a high level of root formation in microshoot cultures in the flowering shrub *Thryptomene ericaea* F.Muell. has been developed. Microshoot cultures were initiated from field grown plants and proliferated in Damiano nutrient medium supplemented with 1.0 µM 6-benzyladenine (BA) and 1.0 µM kinetin. Adventitious rooting was induced by culturing elongated shoots in 0.7% (w/v) water-agar supplemented with 1.0 mM indole-3-butyric acid (IBA) for 48 hours then transferring the shoots to hormone free basal medium for 8 weeks. Under these conditions, a rooting frequency of 85% was achieved. Plantlets were successfully acclimatised in soil, and subsequently developed into phenotypically normal plants.

Keywords: Myrtaceae, *Thryptomene*, root formation, cultures.

Introduction

Thryptomene ericaea F.Muell. is a showy medium sized shrub that grows in the understory of mallee vegetation and is endemic to Kangaroo Island and Eyre Peninsula in South Australia. Growing to a height of 1.2 metres, it produces sprays of flowers between September and December. Flowers include 5 stamens, 5 sepals, 5 petals and 2 ovules. Petals are ovate, white in colour and approximately 1 mm long (Green 1986). A clonal selection, Centenary Starburst™ (Scarvelis 2000), was chosen as South Australia's floral emblem for the Centenary of Federation in 2001. There has also been interest in the use of *Thryptomene ericaea* in the floral industry, as the flowering branches have long vase life and are excellent as cut flowers (Beardsell 1996). Currently, flowering stems are harvested from wild populations on Kangaroo Island, South Australia. Although the present volume of harvested material is not significant, there is potential for increasing pressures on these wild populations.

Propagation of this woody plant species has proven difficult using conventional methods, with a strike rate of less than 10% reported for woody cuttings (State Flora, South Australia, personal communication). Whilst germination of seed from *Thryptomene* species has been reported, germination has proven difficult, with seeds requiring specific pretreatments to overcome dormancy issues (Beardsell et al. 1993). Several attempts have also been made to tissue culture shoot tips of a closely related species, *Thryptomene calycina* (Lindl.) Stapf, but endogenous contaminants prevented establishment of *in vitro* shoot cultures (Beardsell 1996). To our knowledge this is the first report describing successful root formation in microshoot cultures for the genus.

Materials & Methods

Research to develop a rooting protocol was conducted over a 12 month period between January and December 2000 at the Botanic Gardens of Adelaide (South Australia). Shoot material of *Thryptomene ericaea* was collected during spring 1999 from adult plants growing at Wittunga Botanic Garden (South Australia). Shoot material harvested outside the plants active growing season was found to be suboptimal for introduction into tissue culture due to increased levels of endogenous contamination issues (data not shown). Shoot pieces were excised in the laboratory and washed in 0.4% (v/v) Physan 20 (a mixture of benzyl ammonium chlorides) for 2 minutes, then rinsed 3 times with sterile distilled water. The final rinse occurred overnight (12 hours) with gentle agitation. Explants, 10–15 mm in length, consisting of shoot tips and nodal segments bearing small axillary shoots were excised from the washed material. These were surface sterilised in 0.015% (w/v) mercuric chloride dissolved in 0.4% (v/v) sodium hypochlorite for 9 minutes (Rugini 1986) then rinsed 3 times with sterile distilled water. Sterilised explants were cultured in individual 30 ml polycarbonate tissue culture tubes containing ½-strength Damiano nutrient medium (Damiano et al. 1991) supplemented with 0.5 µM 6-benzyladenine (BA). This nutrient medium, is based on Murashige and Skoog (1962) medium, and has been modified by lowering the total nitrogen to approximately ½-strength

Fig. 1. Root induction in microshoot cultures of *Thryptomene ericaea*. For root induction, explants were cultured in 0.7% (w/v) water-agar supplemented with 1.0 mM IBA for 48 hours then transferred to full-strength hormone free Damiano medium for 8 weeks. Scale bar 10 mm.

and changing the ratio of NO_3:NH_4 to 3:1 (Damiano et al. 1991). All media contained 3.0% (w/v) sucrose, were solidified with 0.7% (w/v) agar (SIGMA) and adjusted to pH 5.7 prior to autoclaving (121°C at a pressure of 103 kPa for 20 minutes). Explants were maintained at 25 ± 1°C with a 16 hour photoperiod (40 µmol m-2 s-1) provided by Osram 36 W cool white fluorescent tubes. After 4 weeks, healthy uncontaminated explants were transferred to full-strength Damiano basal medium supplemented with 1.0 µM BA + 1.0 µM kinetin, and maintained with a monthly subculture period. Explants were subcultured at least 3 times on this medium before being used for rooting experiments.

Three rooting experiments were conducted with methods based on earlier work undertaken by the author (Ainsley et al. 2001). The first tested chronic auxin application over an 8 week period. Shoots were transferred to rooting media supplemented with indole-3-butyric acid (IBA: 0.0, 1.0, 2.5, 5.0, 10.0, 15.0 µM), α-naphthaleneacetic acid (NAA: 0.0, 1.0, 2.5, 5.0, 10.0, 15.0 µM) or combinations of both (0.5+0.5, 1.25+1.25, 2.5+2.5, 5.0+5.0, 7.5+7.5 µM). The second experiment examined the effectiveness of exposing shoots to acute auxin levels by dipping explants in different hormone solutions. Shoots were dipped for 30 seconds in either IBA (0.0, 5.0, 10.0 mM) or NAA (0.0, 5.0, 10.0 mM) that had been dissolved in 70% (v/v) ethanol, followed by a transfer to hormone free basal medium for 8 weeks. The final experiment tested a similar approach, using lower auxin concentrations for longer time periods. In this experiment, shoots were cultured in water-agar (0.7% w/v) supplemented with IBA (0.0, 0.5, 1.0 mM) or NAA (0.0, 0.5, 1.0 mM) for a range of times (24, 48, 72 hours) before being transferred to hormone free basal medium for a further 8 weeks. Full-strength Damiano medium was used for all rooting experiments. For each treatment, 20 elongating shoots (10-15 mm in length) were tested individually in 30 ml polycarbonate tissue culture tubes. Explants were cultured under dark conditions for the first 7 days, and then transferred to light conditions (described above). On conclusion of experiments, rooting frequency was determined as a percentage of the number of explants that developed roots for each treatment. Root numbers were counted at the same time, with a standard error determined within treatments. Experiments were replicated once only.

To ensure rooted explants could be outplanted and were phenotypically normal, shoots with roots 5 − 10 mm in length were transferred to a glasshouse. Plantlets were removed from culture, and the roots gently washed in distilled water to remove any residual agar medium. These were planted into seedling trays containing sterilised compost (composted pine bark: washed sand: perlite: peat = 6:2:1:1) and placed inside large clear plastic bags which were sealed to maintain high humidity. Over 4 weeks, relative humidity was slowly decreased by gradually opening the plastic bags. After a further 6-8 weeks, established plants were transferred to individual 100 mm plastic pots. Plantlets were acclimatised in a glasshouse at 25 ± 2°C under natural daylight conditions.

Results & Discussion

IBA was the most effective auxin for the initiation of adventitious roots, with the acute auxin treatments yielding higher rooting frequencies and root numbers as compared to the chronic auxin treatments (Table 1). Although some root induction was recorded following shoot exposure to IBA or NAA at levels up to 15.0 µM for 8 weeks, the maximum rooting frequency with this approach was only 30% (Table 1). Furthermore, there appeared to be no advantage in combining the two auxins in the rooting medium. In comparison, while rooting frequencies of up to 50% (Table 1) occurred following explant dipping in concentrated auxin solutions (5.0−10.0 mM), this technique induced explant burning and high levels of shoot hyperhydricity. The most suitable approach occurred by lowering auxin concentration (0.5−1.0 mM) and extending exposure time for up to 72 hours. Using this regime, up to 95% of explants developed adventitious roots (Table 1). Optimum rooting conditions were achieved by culturing shoots on 0.7% (w/v) water-agar supplemented with 1.0 mM IBA for 48 hours then transferring shoots to hormone free basal medium for 8 weeks. Under these conditions, 85% of explants developed multiple roots that were branched and white in colour (Figure 1). Whilst a higher level of rooting occurred by pretreating shoots with 1.0 mM IBA for 72 hours (Table 1), shoot health was reduced, and was therefore not deemed optimum.

On transfer to the glasshouse, 70% of the plantlets transferred survived acclimatisation and developed into phenotypically normal plants. Flowering was observed within 3 years.

This study reports a protocol for rooting *Thryptomene ericaea* under tissue culture conditions. These techniques provide a rapid and more efficient alternative to the

Table 1. Summary of auxin treatments that induced adventitious rooting in *Thryptomene ericaea*.

Auxin	Concentration (µM)	Exposure time	Exposure method	Rooting frequency (%)*	Number of roots / explant (± SE)
Chronic					
IBA	15	8 wks	solid media	15	1.0 ± 0.0
NAA	5	8 wks	solid media	30	1.0 ± 0.0
NAA	15	8 wks	solid media	20	1.5 ± 0.3
IBA + NAA	2.5 + 2.5	8 wks	solid media	10	1.0 ± 0.0
IBA + NAA	5 + 5	8 wks	solid media	10	1.5 ± 0.0
IBA + NAA	7.5 + 7.5	8 wks	solid media	15	1.0 ± 0.0
Acute					
IBA	5000	30 s	dip treatment	10	4.0 ± 0.0
IBA	10000	30 s	dip treatment	50	1.4 ± 0.3
NAA	5000	30 s	dip treatment	20	1.0 ± 0.0
NAA	10000	30 s	dip treatment	20	2.5 ± 0.5
IBA	500	24 h	solid media	20	1.5 ± 0.5
IBA	500	48 h	solid media	55	2.2 ± 0.4
IBA	500	72 h	solid media	30	1.8 ± 0.3
IBA	1000	24 h	solid media	40	2.9 ± 0.6
IBA	1000	48 h	solid media	85	3.4 ± 0.4
IBA	1000	72 h	solid media	95	4.8 ± 0.4
NAA	500	24 h	solid media	10	1.0 ± 0.0
NAA	500	72 h	solid media	5	2.0 ± 0.0
NAA	1000	24 h	solid media	5	3.0 ± 0.0
NAA	1000	48 h	solid media	10	1.5 ± 0.5
NAA	1000	72 h	solid media	40	2.4 ± 0.5

*based on 20 explants.

methods currently used to propagate this species, and provide an opportunity to produce material for plantation growing to reduce the pressure on natural stands that are currently harvested for the cut flower trade.

Many native Australian plants have proven difficult to root by both woody cuttings and in tissue culture (Johnson 1996). The technique described in this study has since been successfully used to assist with adventitious rooting in *Eucalyptus* species (Glocke et al. 2006) and may have direct application for other native Australian woody plant species where rooting is proving difficult.

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