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Micropropagation of the therapeutic-honey plants *Leptospermum polygalifolium* and *L. scoparium* (Myrtaceae)

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Abstract. Demand for therapeutic honey is driving establishment of *Leptospermum* plantations. This study developed micropropagation methods for two species – *Leptospermum polygalifolium* Salisb. and *L. scoparium* J.R.Forst. & G. Forst. The study determined how shoot proliferation and adventitious rooting were influenced by the original explant position on the seedling and the concentration of benzyladenine (BA) in the proliferation medium. Hormone-free node culture was highly effective for both species. Nodal explants often formed roots in the absence of BA and developed elongated axillary shoots. Median shoot numbers of 584 and 659 were formed in 31–32 weeks from a single *L. polygalifolium* or *L. scoparium* seed, respectively. A low BA dose was effective for callogenesis and shoot proliferation of *L. polygalifolium*, but not *L. scoparium*. The median number of shoots produced from a single *L. polygalifolium* seed was 630 using 2.22-μM BA. This dose induced extremely high shoot numbers in some clones because explants often produced extensive callus and multiple short shoots. Shoots formed adventitious roots without indole-3-butyric acid and plantlets were acclimatised to nursery conditions. The original explant position did not influence shoot proliferation or adventitious rooting. *Leptospermum polygalifolium* and *L. scoparium* proved amenable to micropropagation, facilitating rapid establishment of nectar plantations.

Keywords: adventitious rooting, auxin, cytokinin, dihydroxyacetone, manuka honey, methylglyoxal, Myrtaceae, propagation, therapeutic honey, tissue culture, topophysis, *Leptospermum*.

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Introduction

Mānuka honey has been harvested traditionally in New Zealand from hives of honeybees that forage on the mānuka tree, *Leptospermum scoparium* (Morgan *et al*. 2019; Bong *et al*. 2021; Schmidt *et al*. 2021). Mānuka honey contains high levels of the antimicrobial and wound-healing compound, methylglyoxal (MGO), which is converted gradually in the honey from dihydroxyacetone (DHA) that is found in *L. scoparium* nectar (Carter *et al*. 2016; Cokcetin *et al*. 2016; Grainger *et al*. 2016; Niaz *et al*. 2017; Schmidt *et al*. 2021). Honeybees that forage on *L. scoparium* and some other *Leptospermum* species in Australia also produce honey that contains high levels of MGO (Cokcetin *et al*. 2016; Pappalardo *et al*. 2016; Williams *et al*. 2018). Demand for therapeutic MGO-containing honey is greater than supply, so there is a drive to establish *Leptospermum* plantations to provide nectar for therapeutic honey production.

One of the limitations to plantation establishment has been the difficulty in propagating *Leptospermum* species as seedlings because the seed can be difficult to extract from the fruit or difficult to germinate (Shipton and Jackes 1986; Lyne and Crisp 1996; Battersby *et al*. 2017). Therefore, clonal propagation methods have been sought for propagating *Leptospermum* species by tissue culture and cuttings. Clonal propagation has been used to propagate limited numbers of elite clones in clonal plantation programs (Trueman 2006; Xavier *et al*. 2013; Trueman *et al*. 2018). These programs have the advantage that individual clones have been selected for desirable mature-age characteristics such as trunk straightness, wood volume or fruit yield (Aimers-Halliday and Burdon 2003; Mitchell *et al*. 2004; Wendling *et al*. 2014*a*, 2014*b*). Clonal plantation programs have the disadvantage that selected clones have often undergone maturation, reducing their propagation capacity and growth potential (Aimers-Halliday and Burdon 2003; Pijut *et al*. 2011; Wendling *et al*. 2014*a*, 2014*b*). Increasingly, clonal propagation is used instead to propagate multiple clones that are the progeny of selected mother trees in 'vegetative family plantation programs' (Lee 2007; Trueman *et al*. 2018). These programs have the disadvantage that not all clones share the full suite of desirable characteristics of the selected mother tree.

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However, a vegetative family program has the advantage that the clones are juvenile, often giving them much higher propagation capacity and growth potential than mature clones (McMahon *et al*. 2013, 2014; Wendling *et al*. 2014*a*, 2014*b*).

The current study is part of a program focussed on developing clonal propagation methods for juvenile germplasm of therapeutic-honey *Leptospermum* species. Little was known about the micropropagation capacity of most *Leptospermum* species, although an extensive array of micropropagation methods has been developed for other Myrtaceae species from the eucalypt genera, *Corymbia* and *Eucalyptus*. These methods employ cytokinins, typically benzyladenine (BA), to promote axillary bud outgrowth or adventitious shoot production (Trueman *et al*. 2018). Shoots of *L. scoparium* and its hybrids have been proliferated *in vitro* in full-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) containing BA at 0.00–4.44 μM (Braun and Leung 1991; Seelye *et al*. 2001; Fan *et al*. 2013). Acclimatisation of plantlets to nursery conditions proved difficult for *L. scoparium* (Braun and Leung 1991) but was successful for plantlets of *L. scoparium* × *L. rotundifolium* backcrossed to *L. rotundifolium* (Seelye *et al*. 2001). Eucalypt shoots are usually proliferated in full-strength MS media or MS salts that are supplemented with BA at 0.44–6.67 μM (Niccol *et al*. 1994; Hervé *et al*. 2001; Glocke *et al*. 2005; Arya *et al*. 2009; Hung and Trueman 2012). However, eucalypt shoot proliferation can sometimes be prolific in media lacking BA (Brondani *et al*. 2013; Trueman *et al*. 2018). Hormone-free medium promotes extensive shoot elongation and the production of multiple nodes, whereas media containing BA promote callogenesis and the production of multiple short shoots, in cultures of *Corymbia torelliana* × *C. citriodora* (Trueman and Richardson 2007; Hung and Trueman 2010). Shoot proliferation potential in the hormone-free medium was influenced by the position on the seedling from which the original explant was taken, with proliferation being higher from the first node (i.e. cotyledonary node) and second node than more-apical nodes (Hung and Trueman 2011). These types of positional effects on the growth and development of propagules are termed 'topophysis', and an understanding of topophysic effects can lead to great improvements in the efficiency of a propagation system (George 1993; Mitchell *et al*. 2004; Hung and Trueman 2011; Wendling *et al*. 2015).

This study aimed to develop micropropagation methods for two species – *L. polygalifolium* and *L. scoparium* – that are highly sought for establishing therapeutic honey plantations. The study aimed to determine the extent to which shoot proliferation and nursery acclimatisation are influenced by the concentration of BA in the proliferation medium. The study also aimed to assess the extent to which micropropagation capacity is affected by the position of the initial explant on the original seedling.

Materials and methods

Seed germination

Seeds of a local subtropical provenance of *L. polygalifolium* were provided by Dr Peter Brooks (University of the Sunshine Coast, Sippy Downs, Australia) and seeds of selected *L. scoparium* from southern Australia were provided by Mr Ted Allender (ERA Nurseries, Hamilton, Australia). Batches of ~100 seeds of each species were washed in 70% ethanol (v/v) for 1 min in 70-mL vials containing one drop of Tween 20. They were then rinsed in sterile distilled water. Each batch was then separated into batches of 25 seeds, and each new batch was transferred into a new vial containing 1% sodium hypochlorite with one drop of Tween 20. The vials were swirled on an orbital shaker at 240 rpm for 10 min. The seeds were rinsed in sterile distilled water. Seeds were placed on sterile paper to remove excess liquid between solutions. Seeds were plated (25 seeds per 90-mm-diameter Petri dish) onto germination medium consisting of half-strength MS basal salts (PhytoTechnology Laboratories, Shawnee Mission, KS) with 58.4-μM sucrose, solidified with 8 g L^{-1} of agar (Bacto Laboratories, Liverpool, Australia) and with pH adjusted to 5.8 before autoclaving at 121°C for 20 min. This procedure was repeated to create 90 dishes, each of 25 seeds, per species. The seeds were maintained at 28°C under a 16-h photoperiod (~100 μmol m^{-2} s^{-1} with fluorescent tubes) for 3–4 weeks.

Shoot induction (first passage)

All shoots greater than 5-mm length were excised at the root collar and transferred (5 shoots per jar) into 375-mL glass jars containing 50 mL of shoot induction medium consisting of full-strength MS medium with vitamins (PhytoTechnology Laboratories) with 87.6-μM sucrose, solidified with 8 g L^{-1} of agar and with pH adjusted to 5.8 before autoclaving at 121°C for 20 min. The shoots were maintained at 28°C under a 16-h photoperiod (~100 μmol m^{-2} s^{-1} with fluorescent tubes) for 5 weeks. The shoots were then dissected into their individual nodes by cutting immediately above each node (Fig. 1*a*). The apical bud was regarded as a single node.

Shoot proliferation (second, third and fourth passages)

Each node from each seedling was transferred to a 375-mL glass jar containing full-strength MS medium with vitamins, with 87.6-μM sucrose and one of the following five hormone concentrations: 0.0-, 2.2-, 4.4-, 8.9- or 17.8-μM BA (Sigma, Saint Louis, MO, USA). All nodes from a single seedling were placed in the one jar (i.e. one clone per jar) for the first proliferation passage. The original position of each node on the seedling was marked on the outside of the jar, with '1' being the cotyledonary node and '2,' '3', '4', 5' or '6' representing progressively more-apical nodes. At least 15 clones per BA treatment were available for each species, and each clone number was marked on the outside of the jar. The shoots were maintained at 28°C under a 16-h photoperiod (~100 μmol m^{-2} s^{-1} with fluorescent tubes). The shoots were maintained for 7 weeks, then dissected into their nodes and transferred to fresh medium of the same BA concentration for a second proliferation passage of 8 weeks. They were then dissected into their nodes and transferred again to fresh medium of the same BA concentration for a third proliferation passage of 8 weeks. The number of shoots with roots and the number of nodes available for transfer from each of the original-seedling node positions was counted

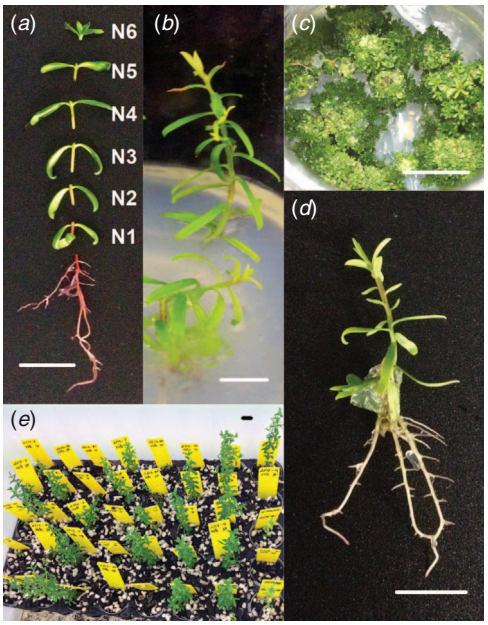

Fig. 1. Micropropagation of *Leptospermum polygalifolium and L. scoparium*. (*a*) *In-vitro* seedling dissected into six nodes (N1–N6) for transfer to proliferation medium; (*b*) *L. polygalifolium* shoot forming adventitious roots (at base) and elongating axillary shoots in the absence of benzyladenine (BA); (*c*) *L. polygalifolium* producing multiple short shoots from or through callus in medium containing 2.22-μM BA; (*d*) *L. polygalifolium* shoot that has formed three adventitious roots in medium lacking indole-3-butyric acid; (*e*) *L. scoparium* plantlets that have been acclimatised to nursery conditions. Scale bars: 2 cm.

at the end of each proliferation passage, i.e. after 7, 15 and 23 weeks in proliferation medium. The percentage of shoots per clone with roots and the number of available nodes per clone was then calculated from the data obtained from each of the original-seedling node positions.

Root induction

A random subsample of 20 shoots >15 mm long (where available) in hormone-free medium was taken from each original seedling-node position of each clone after the third proliferation passage. These shoots were transferred randomly into one of four glass jars (five shoots per jar) containing 50 mL of half-strength MS medium with 58.4-μM sucrose, 8 g L^{-1} of agar and either 0.0-, 4.9-, 19.6- or 78.4-μM indole-3-butyric acid (IBA). The shoots were maintained in this medium for 24 h in darkness at 24°C. They were then transferred to hormone-free half-strength MS medium with 58.4-μM sucrose and 8 g L^{-1} of agar, where they were maintained for 6 days in darkness and then 3 weeks at ~100 μmol m^{-2} s^{-1} irradiance. The same process was applied to shoots from proliferation medium containing 2.2-μM BA, except that these shoots were transferred first to hormone-free proliferation medium for 5 weeks to allow shoot elongation before they were transferred to the IBA treatments.

Nursery acclimatisation

Plantlets were then transferred into 70-mL propagation tubes (Darby *et al*. 2021) containing eucalypt seedling mix consisting of a 75/25 (v/v) mixture of shredded pine bark and perlite, with 3 kg of 8–9-month slow-release Osmocote fertiliser (Scotts International, Heerlen, Netherlands), 3 kg of lime (Unimin, Lilydale, Australia), 1 kg of gypsum (Queensland Organics, Narangba, Australia), 1 kg of Micromax micronutrients (Scotts Australia, Baulkham Hills, Australia) and 1 kg of Hydroflow wetting agent (Scotts Australia) incorporated per square metre (Trueman *et al*. 2013*a*, 2013*b*, 2013*c*, 2013*d*, 2014). The plantlets were maintained at 28°C under a 16-h photoperiod (~50 μmol m^{-2} s^{-1}) in a sealed 80-L plastic tub for a further 4 weeks. The tubs were then transferred to a polyethylene propagation chamber that was custom-built within a glasshouse. Plantlets were maintained within the sealed tub and under 50% shadecloth (~85 μmol m^{-2} s^{-1}) for 3 days, and then the tub lid was opened progressively at 4-day intervals and the shadecloth removed to increase irradiance to ~380 μmol m^{-2} s^{-1} over a further 12 days. Mist irrigation was provided for 1 min every 10 min from 0700 to 1800 hours. The percentage of shoots from each jar that had survived and formed roots was recorded after 8 weeks in the glasshouse.

Statistical analyses

Shoot proliferation data was analysed by Kruskal–Wallis test because shoot number data was not normally distributed. Dunn's tests with Bonferroni corrections were used when significant differences among the medians were detected by Kruskal–Wallis test. Medians are presented with 25th and 75th percentiles, 10th and 90th percentiles, and outliers. Rooting data was analysed by *t*-test or by random block ANOVA because adventitious rooting data was normally distributed. Clones were regarded as blocks. Means are presented with standard errors. Differences between medians or means were regarded as significant at $P < 0.05$.

Results

Shoot proliferation

The highest production of both *L. polygalifolium* and *L. scoparium* shoots during the first proliferation passage occurred in hormone-free medium (Fig. 2*a*, *b*). Explants developed new nodes on elongating axillary shoots in the absence of BA (Fig. 1*b*). *L. polygalifolium* also produced multiple shoots during the second and third proliferation passages in medium containing 2.22-μM BA (Fig. 2*c*, *e*), with these being short shoots that developed either from or through callus (Fig. 1*c*). Extensive explant death occurred in media containing 4.44–17.78-μM BA for *L. polygalifolium* (Fig. 2*c*, *e*) and 2.22–17.78-μM BA for *L. scoparium* (Fig. 2*d*, *f*). As a result, the most effective BA concentrations for shoot proliferation were 0.00 or 2.22 μM for *L. polygalifolium* (Fig. 2*e*) and 0.00 μM for *L. scoparium* (Fig. 2*f*). The median (range) numbers of shoots produced per clone were 584 (0–5427) in 0.0-μM BA and 630 (0–58 635) in 2.2-μM BA for *L. polygalifolium* and 659 (0–5985) in 0.0-μM BA for *L. scoparium*.

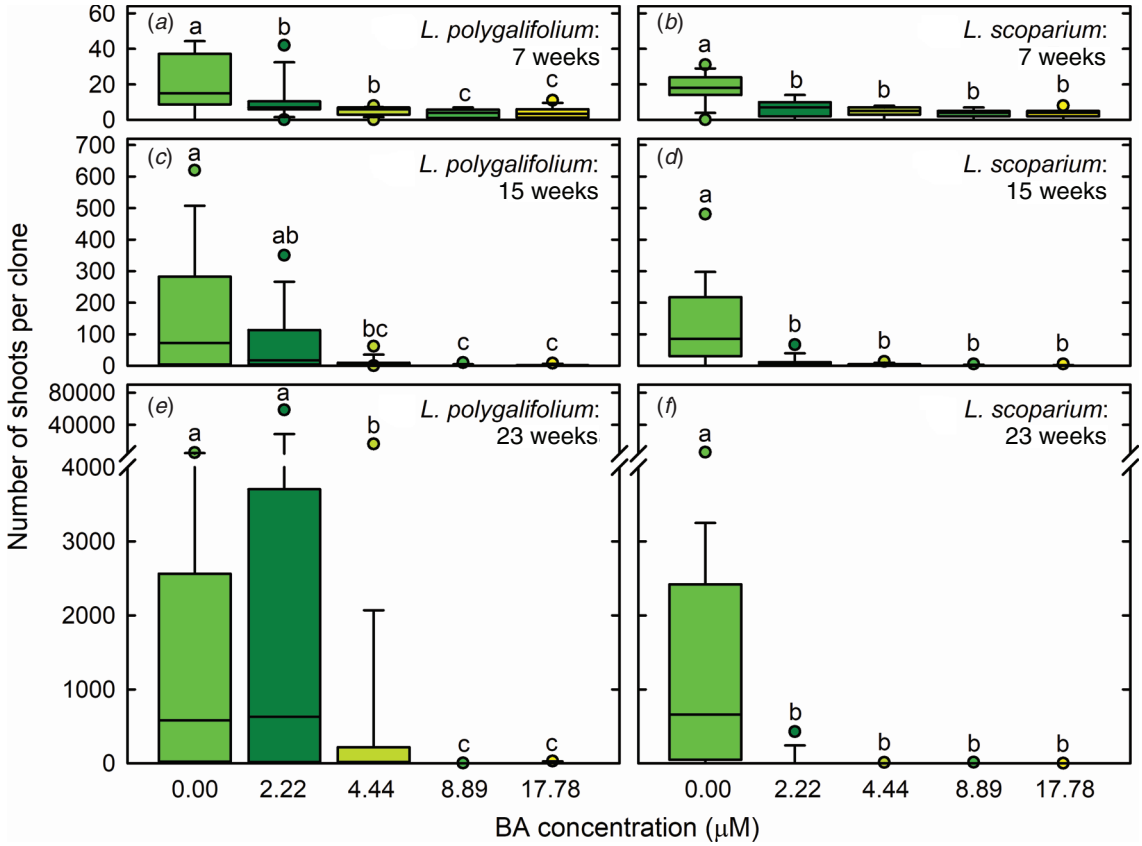

Fig. 2. Effect of benzyladenine (BA) concentration on the number of shoots produced per clone of (*a*, *c*, *e*) *Leptospermum polygalifolium* or (*b*, *d*, *f*) *L. scoparium* after (*a*, *b*) 7 weeks, (*c*, *d*) 15 weeks and (*e*, *f*) 23 weeks in proliferation medium. Medians are presented with 25th and 75th percentiles (boxes), 10th and 90th percentiles (whiskers), and outliers. Medians with different letters within a species and time point are significantly different (Kruskal–Wallis and Dunn's test with Bonferroni corrections, $P < 0.05$, n = 15–19 clones).

Root induction and nursery acclimatisation

Extensive shoot death occurred when *L. polygalifolium* and *L. scoparium* were treated with 4.9-, 19.6- or 78.4-μM IBA (data not presented). However, rooting occurred in medium that lacked IBA (Fig. 1*d*), and many of these plantlets were acclimatised successfully to nursery conditions (Fig. 1*e*). The percentages of shoots that formed roots in the absence of IBA and acclimatised successfully to nursery conditions were 31 ± 6 and 30 ± 9% for *L. polygalifolium* shoots that had been proliferated in media containing 0.00- or 2.22-μM BA respectively (Fig. 3*a*). The percentages of shoots that formed roots and acclimatised successfully were 44 ± 9 and 29 ± 10% for *L. scoparium* shoots that had been proliferated in media containing 0.00- or 2.22-μM BA respectively (Fig. 3*b*).

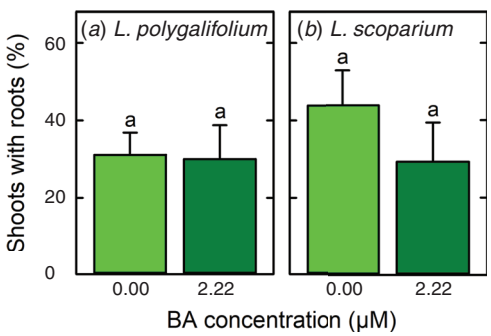

Fig. 3. Effect of benzyladenine (BA) concentration in the proliferation medium on the percentage of (*a*) *Leptospermum polygalifolium* or (*b*) *L. scoparium* shoots that subsequently formed roots in hormone-free medium and had survived after 8 weeks in the laboratory and 8 weeks acclimatising in the glasshouse. Means (+s.e.) within a species do not differ significantly (*t*-test, $P > 0.05$, n = 11–15 clones).

Topophysic effects on proliferation, rooting and nursery acclimatisation

The position of the initial explant on the original seedling did not significantly affect the proliferation capacity of *L. polygalifolium* or *L. scoparium* shoots in media containing either 0.00- or 2.22-μM BA (Fig. 4). Many shoots formed roots in proliferation medium containing 0.00-μM BA (Table 1) but rooting was almost never observed in media containing BA (data not shown). Root formation in the hormone-free proliferation medium was not affected significantly by the original explant position (Table 1). Root induction and nursery acclimatisation of

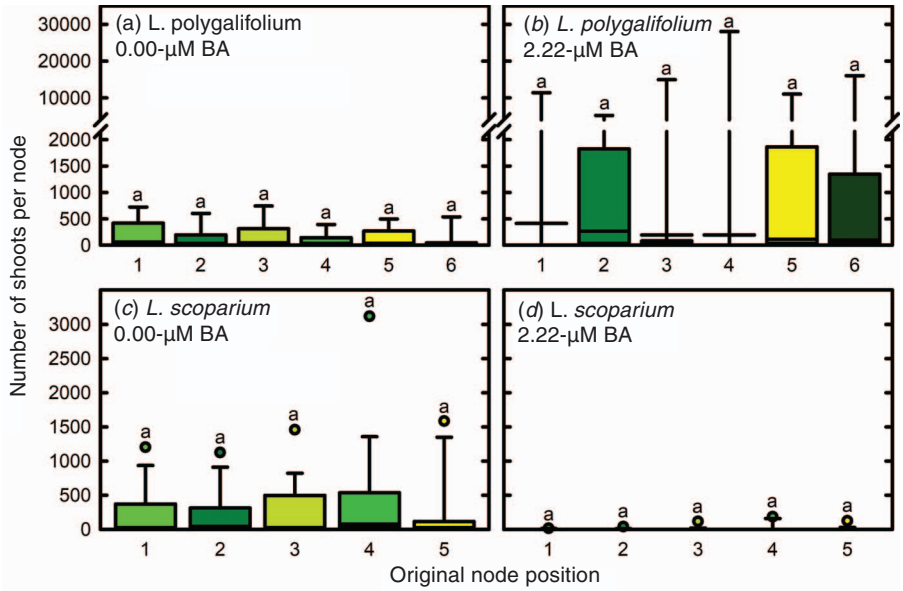

Fig. 4. Influence of the original node position on the number of shoots produced per seedling node of (*a*, *b*) *Leptospermum polygalifolium* or (*c*, *d*) *L. scoparium* after 23 weeks in proliferation medium containing (*a*, *c*) 0.00-µM benzyladenine (BA) or (*b*, *d*) 2.22-µM BA. Medians are presented with 25th and 75th percentiles (boxes), 10th and 90th percentiles (whiskers), and outliers. Medians within a species and BA concentration do not differ significantly (Kruskal–Wallis test, $P > 0.05$, n = 8–19 clones).

Table 1. Influence of the original seedling node position on the percentage of *Leptospermum polygalifolium* and *L. scoparium* shoots that formed roots after 7, 15 and 23 weeks in hormone-free proliferation medium

Means (±s.e.) within a species and period in proliferation medium do not differ significantly (random block ANOVA, $P > 0.05$, n = 5–17 clones)

Period in proliferation medium	Node position (1 = cotyledonary; 5 or 6 = apical)					
	1	2	3	4	5	6
L. polygalifolium						
7 weeks	20 ± 10	7 ± 4	14 ± 9	16 ± 9	10 ± 6	23 ± 7
15 weeks	67 ± 21	47 ± 20	40 ± 22	40 ± 22	25 ± 16	27 ± 16
23 weeks	20 ± 7	23 ± 12	32 ± 17	24 ± 13	17 ± 7	8 ± 4
L. scoparium						
7 weeks	16 ± 4	14 ± 3	28 ± 5	29 ± 7	19 ± 5	–
15 weeks	48 ± 13	26 ± 10	26 ± 9	38 ± 11	36 ± 13	–
23 weeks	24 ± 8	23 ± 6	20 ± 6	31 ± 8	29 ± 11	–

plantlets was also not influenced significantly by the original explant position (Table 2).

Discussion

Seedling shoots of *L. polygalifolium* and *L. scoparium* proved highly susceptible to shoot death in the presence of BA doses, 4.44–17.78 and 2.22–17.78 µM respectively that are commonly used for proliferation of eucalypt shoots. These doses are used for shoot proliferation of eucalypts including *C. citriodora*, *C. torelliana* × *C. citriodora*, *Eucalyptus cloeziana*, *E. dunnii*, *E. erythronema* and *E. stricklandii* (Tanabe *et al*. 1996; Glocke *et al*. 2005; Hung and Trueman 2012; Navroski *et al*. 2014; Oliveira *et al*. 2015; Trueman *et al*. 2018). However, hormone-free medium was highly effective for shoot proliferation of both *L. polygalifolium* and *L. scoparium*. Nodal explants often formed roots in this medium and they developed elongated axillary shoots from existing axillary buds. As a result, median shoot numbers of 584 and 659 could be formed in 31–32 weeks from plating of a single *L. polygalifolium* or *L. scoparium* seed, respectively. This type of node culture in BA-free medium has also proven highly effective for propagation of *C. torelliana* × *C. citriodora* (Trueman and Richardson 2007; Hung and Trueman 2010). A potential advantage of node culture is that shoot proliferation is not associated with the formation of callus, and so this method reduces the risk of releasing or inducing somaclonal variation in the propagated plant population (George 1993).

Use of a low dose of BA (2.22 µM) was also effective for shoot proliferation of *L. polygalifolium*, but not *L. scoparium*. The median number of shoots produced from a single *L. polygalifolium* seed in this medium (630) was similar to the number produced in BA-free medium (i.e. 584). However, BA had the capacity to induce extremely high numbers of shoots in some clones. This was because explants in BA-containing

Table 2. Influence of the original seedling node position on the percentage of *Leptospermum polygalifolium* and *L. scoparium* shoots that formed roots in hormone-free medium and had survived after 8 weeks in the laboratory and 8 weeks acclimatising in the glasshouse

Means (±s.e.) within a species and proliferation medium do not differ significantly (random block ANOVA, $P > 0.05$, $n = 3–12$ clones)

[Benzyladenine] in proliferation medium	Node position (1 = cotyledonary; 5 or 6 = apical)					
	1	2	3	4	5	6
L. polygalifolium						
0.00 µM	33 ± 9	32 ± 16	61 ± 16	30 ± 12	33 ± 15	61 ± 15
2.22 µM	–	21 ± 10	16 ± 10	52 ± 22	29 ± 12	40 ± 25
L. scoparium						
0.00 µM	20 ± 8	46 ± 13	42 ± 13	50 ± 12	38 ± 12	–
2.22 µM	17 ± 8	50 ± 22	54 ± 27	46 ± 18	41 ± 21	–

medium often produced extensive callus and multiple short shoots, without forming roots. Similar callogenesis has been observed in cultures of *C. torelliana* × *C. citriodora* with the same BA dose (Trueman and Richardson 2007; Hung and Trueman 2010, 2011, 2012). Shoot formation appeared to occur through a combination of axillary shoot production, callogenesis and shoot regeneration, as it does in *C. torelliana* × *C. citriodora* (Trueman and Richardson 2007; Hung and Trueman 2010). The relative contributions of axillary shoots and adventitious shoots to total shoot proliferation are difficult to ascertain, especially because many Myrtaceae species have multiple axillary and accessory buds within each leaf axil (Burrows *et al*. 2008; Burrows 2013). Histological examination would be required to determine the cellular origin of the multiple shoots produced in the presence of BA (Dobrowolska *et al*. 2017; Trueman *et al*. 2018). The possibility that many of the shoots are formed via an intervening callus phase may explain the very high shoot proliferation in some clones. However, it also means that this method has the potential to release or induce somaclonal variation (George 1993; Tibok *et al*. 1995; Mo *et al*. 2009). This might not be a concern in a vegetative family propagation program that establishes nursery stock populations and plantations with a large and diverse range of juvenile clones.

Shoots of both *L. polygalifolium* and *L. scoparium* were converted successfully into plantlets and acclimatised to nursery conditions. Rooting and acclimatisation success were similar among shoots that had been produced along the hormone-free node-culture pathway and the BA-induced callogenic pathway, noting, though, that shoots from the latter pathway were maintained in the absence of BA for 5 weeks before root induction. The percentages of shoots that formed roots and acclimatised successfully were somewhat low (30–31% for *L. polygalifolium* and 29–44% for *L. scoparium*), and these percentages could not be increased using IBA doses that stimulate adventitious rooting in eucalypt shoots (Trueman *et al*. 2018) and in shoots of *L. scoparium* × *L. rotundifolium* backcrossed to *L. rotundifolium* (Seelye *et al*. 2001). Fortunately, the nursery stock plants formed from the *L. polygalifolium* and *L. scoparium* plantlets have very high capacity for rooted-cutting production (Darby *et al*. 2021). The stock plants produce shoots prolifically and the cuttings from these shoots have very high capacity for adventitious rooting. Rooting percentages of *L. polygalifolium* and *L. scoparium* cuttings are 88–93 and 65–76% respectively, which would be considered high for most eucalypt species (Trueman *et al*. 2017; Darby *et al*. 2021). These rooting percentages are also higher than, or similar to, the 70% threshold that is required by many commercial nurseries (Trueman 2006; Hunt *et al*. 2011; Rigby and Trueman 2015).

No evidence was found of topophysic effects on *in-vitro* rooting, shoot proliferation or *ex vitro* acclimatisation of *L. polygalifolium* or *L. scoparium* shoots. The first (cotyledonary) node and the second node have higher rooting, shoot elongation and shoot proliferation capacity than more apical nodes in hormone-free node cultures of *C. torelliana* × *C. citriodora* (Hung and Trueman 2011). Such topophysic effects are not evident in BA-induced callogenic cultures, where rooting and extensive shoot elongation do not occur. These topophysic effects in *C. torelliana* × *C. citriodora* cultures allow the separation of different seedling nodes into different propagation pathways to optimise shoot proliferation, clonal archiving and field testing. The current results indicate that the first five or six nodes of *L. polygalifolium* or *L. scoparium* have similar proliferation and acclimatisation capacity. Therefore, separation of explants on the basis of node position would not be beneficial unless subsequent maturation effects on plant performance, associated with higher node positions, became evident in the nursery or plantation (Aimers-Halliday and Burdon 2003; Mitchell *et al*. 2004; Trueman 2006; Wendling *et al*. 2014*a*, 2014*b*).

In conclusion, *L. polygalifolium* and *L. scoparium* have high capacity for mass propagation in a simple node-culture system that does not employ BA and does not induce callus formation. One of these species, *L. polygalifolium*, also has high capacity for micropropagation in a callogenic-culture system that uses a low dose of BA (i.e. 2.22 µM). Shoots from both systems can be converted into plantlets and acclimatised to nursery conditions. These plantlets have been found to have excellent capacity as nursery stock plants for rooted-cutting production (Darby *et al*. 2021). These results demonstrate that *L. polygalifolium* and *L. scoparium* can be propagated rapidly to establish high-value nectar plantations for therapeutic-honey production.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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