BRIEF COMMUNICATION

Ex vitro rooting of micropropagated shoots of *Stackhousia tryonii*

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Abstract

Micropropagated shoots of *Stackhousia tryonii* were exposed (individually or in combination) to indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthalene acetic acid (NAA) at concentrations 1, 2 or 4 g dm$^{-3}$ with the view to induce rooting under *ex vitro* conditions. The treated microshoots were grown in a mist room for four weeks and assessed for survival, rooting percentage, number of roots and root length. The results showed that IBA at 2 g dm$^{-3}$ was most effective in inducing roots. Mixing of two or more auxins markedly reduced rooting percentage indicating antagonistic effects. The results demonstrated the potential of combining *ex vitro* rooting and hardening in one step, with view to reducing costs of multiplying plants via micropropagation.

*Additional key words*: auxins, indole-3-acetic acid, indole-3-butyric acid, 1-naphthalene acetic acid, microshoots, tissue culture.

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*Stackhousia tryonii* Bailey is a perennial herb that belongs to family *Stackhousiaceae*. This species is classified as a rare species (conservation code 3RC; Briggs and Leigh 1988) because of its limited distribution on serpentine habitats of Central Queensland, Australia (Batianoff *et al.* 1990). *S. tryonii* accumulates nickel up to 40 mg g$^{-1}$ (d.m.) in its shoots (Batianoff and Specht 1992), thus showing a great potential to exploit it in phytoremediation (decontamination of soils of heavy metals; Cunningham and Berti 1993, Kamnev and van der Lelie 2000) and phytomining (extraction of metals using plants; Brooks *et al.* 1998). Success of exploiting this species in any of these processes, however depends on the ability to propagate *S. tryonii*, as large number of plants are required to make phytoremediation/phytomining economically feasible. As propagation via seeds is difficult (N. Ashwath, personal communication), a micropropagation protocol has been established (Bhatia *et al.* 2002).

Micropropagation typically involves three steps: initiation (establishment of cultures), multiplication of established cultures and rooting of microshoots. However, the last step (rooting) can be achieved *ex vitro* in many crop species including strawberry (Borkowska *et al.* 1999), eucalypts (Xavier and Comerio 1997), citrus (Baruah *et al.* 1996), tea (Rajasekaran 1996) and papaya (Kataoka and Inoue 1991, 1992). Information is lacking on *ex vitro* rooting of *Stackhousia tryonii* or of any other species listed in the family *Stackhousiaceae*. The present study was undertaken to assess the rooting ability of *in vitro*-produced microshoots of *S. tryonii* using various auxins under *ex vitro* conditions.

Mature plants of *Stackhousia tryonii* (*Stackhousiaceae*) were obtained from Canoona (50 km northwest of Rockhampton). The plants were carefully excavated along with the roots and soil, transferred to pots and maintained in a glasshouse for 4 weeks.

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*Abbreviations*: IAA - indole-3-acetic acid, IBA - indole-3-butyric acid, NAA - 1-naphthalene acetic acid, BAP - benzylaminopurine, G, medium - Gamborg *et al.* (1968) medium, MS medium - Murashige and Skoog (1962) medium.

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Micropropagation of *S. tryonii* was achieved as follows (see Bhatia et al. 2002): explants from a single mature plant of *S. tryonii* were cut into nodal segments (20 - 25 mm) and surface sterilized with 0.025 % mercuric chloride for 5 min. Treated shoots were rinsed in sterile water and aseptically transferred to tissue culture tubes (25 x 80 mm) containing B5 (Gamborg et al. 1968) basal medium, supplemented with 1 mg dm$^{-3}$ benzylaminopurine (BAP) and 3 % sucrose and solidified with 8 g dm$^{-3}$ Difco agar. Cultures were maintained in a controlled environment room (CER) at temperature of 25 ± 1 °C, 16-h photoperiod (irradiance of 38 μmol m$^{-2}$ s$^{-1}$ using 'cool white' fluorescent tubes; *Sylvania Gro-Lux*, München, Germany) for 4 weeks. The initiated shoots were multiplied on MS (Murashige and Skoog 1962) basal medium supplemented with 1.5 mg dm$^{-3}$ BAP.

Microshoots (approx. 3 - 4 cm in length) were obtained from 4-month-old cultures during multiplication and thoroughly washed under running water to remove any adhering agar. The microshoots were excised and the cut ends were pulse treated by dipping in single or mixed auxin solutions (Table 1) for 15 s. Auxin solutions were prepared by dissolving the auxin powders in 5 % (v/v) ethanol. Untreated microshoots (control) were dipped in 5 % (v/v) ethanol. The treated microshoots were transferred to *Hyco* trays (1.5 - 2 cm deep) containing steam-sterilized river sand:perlite mix (4:1, v/v). For each treatment 24 microshoots were used. The *Hyco* trays were transferred into a mist room maintained at 32 °C with a relative humidity of 90 %.

The microshoots were harvested after four weeks and washed carefully to expose the roots. Percentage survival of shoots, rooting percentage, total number of primary roots, and root length were measured. All microshoots that remained green were considered living and used in calculating rooting percentage. The effects of different treatments were quantified and the data subjected to statistical analysis (SPSS Inc., Chicago, USA).

<table>
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<th>Code</th>
<th>Auxin(s)</th>
<th>Concentration [g dm$^{-3}$]</th>
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<td>Control (no auxin)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>B1</td>
<td>IBA</td>
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</tr>
<tr>
<td>N1</td>
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<td>ABN</td>
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![Fig. 1. Effect of pulse treatment of different auxins on survival and rooting of *Stackhousia tryonii* microshoots. For abbreviations see Table 1.](image)

Microshoots of *S. tryonii* responded differently to varying hormonal treatments as assessed by their survival, rooting percentage and total number and length of primary roots. Microshoot survival was highest (100 %) with 2 g dm$^{-3}$ IBA (Fig. 1). In other treatments survival ranged from 67 % (2 g dm$^{-3}$ IBA + 2 g dm$^{-3}$ NAA) to 92 % (2 g dm$^{-3}$ NAA). Application of other auxins reduced survival rate in treatments where IBA was involved.

Rooting (strike rate) of microshoots was highest (83 %) at 2 g dm$^{-3}$ IBA. A 75 % rooting was observed at 2 and 4 g dm$^{-3}$ IAA and 4 g dm$^{-3}$ IBA, followed by 67 % in 1 g dm$^{-3}$ IBA, and 2 and 4 g dm$^{-3}$ NAA. Control had 58 % rooting and the rest of the treatments had less than 50 % rooting. These results corroborate those of Suksa-Ard et al. (1998) that best rooting (92 - 94 %) and maximum survival (90 - 98 %) of papaya microshoots were at 10 mM IBA. In studies of *ex vitro* rooting of *Vaccinium myrtillus* and *V. pahalaee*, Shibli and Smith (1996) recorded 95 and 60 % rooting after treatment with 4.9 μM IBA or 5.4 μM NAA, respectively. Yu and Reed (1995) demonstrated successful induction of *ex vitro*
rooting in hazelnuts (*Corylus* sp.) by briefly dipping the microshoots in 1 or 5 mM IBA while Kim et al. (1998) showed that overall *ex vitro* rooting of three clones of *Fraxinus pennsylvanica* was better with 1 mM IBA relative to 1 mM NAA.

At concentration 1 g dm⁻³, the three auxins had variable responses, with only IBA increasing rooting over controls but at 2 and 4 g dm⁻³ all hormones induced higher rooting percentage than the controls. However, when two auxins were mixed, the rooting percentage was markedly reduced. These results demonstrate antagonistic effects of hormones on rooting of *S. tryonii* microshoots. A number of commercial rooting hormones (e.g., *Hormex*, *Brooker Chemical Corp.*, North Hollywood, USA; *Rhizopen*, *Hortus USA Corp.*, New York, USA) contain mixed hormones. Our results therefore, suggest need for caution in selecting commercial hormones as rooting of some species may be inhibited by combinations of auxins.

The number of primary roots was significantly higher

![Fig. 2. Effect of pulse treatment of different auxins on number of primary roots (A) and total root length (B) of *Stackhousia tryonii* microshoots. Error bars represent SE. For abbreviations see Table 1.](image)

in 1 and 2 g dm⁻³ IBA and 4 g dm⁻³ NAA than in controls. All other concentrations of auxins and their combinations had very little effect. Best treatments for the production of roots on microshoots was with 1 g dm⁻³ IBA, doubling the number of primary roots (4.25) over control (1.86) (Fig. 2). Kataoka and Inoue (1992) also reported up to 5.2 and 6.8 roots per shoot in papaya treated with 1 or 2.5 g dm⁻³ IBA, respectively. Total root length (31 mm) was highest with 2 g dm⁻³ IBA. All other single treatments had little influence on the total root length. Again, mixing of two or more hormones markedly reduced total root length.

Among the auxins used, best overall performance in terms of rooting ability of microshoots could be ranked as: IBA > IAA > NAA. The use of IBA at concentration 2 g dm⁻³ gave the best results in terms of survival, rooting and total root length and hence is recommended for use in *ex vitro* rooting of *S. tryonii* microshoots.

References


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