Towards an In Vitro Propagation System for *Astelia* Species

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INTRODUCTION

The genus, *Astelia*, which belongs to the Liliaceae family, comprises about 25 species confined mainly to the Pacific region. Thirteen of the species, including all species named in this research note, are endemic to New Zealand (Moore and Edgar, 1976). They are dioecious, mostly short-tufted terrestrial herbs without a significant stem, found from wet lowland to subalpine environments. *Astelia* taxa are generally untroubled by pests and diseases and do not have particular soil type requirements. Leaves mostly range in length from 10 to 80 cm, but some species have leaves up to 3 m long, e.g., *A. chathamica*, *A. fragrans,* and *A. grandis* have long and graceful arching leaves.

The horticultural potential of this genus is now beginning to be realised internationally and a few species, e.g., *A. chathamica* and *A. nervosa,* are being exported for their attractive foliage and for use as hardy amenity plants. Selections have been made from the two species, *A. nervosa* and *A. nivicola*, in which a range of leaf colours (e.g., red and/or bronze), naturally occur (Metcalf, 1993). Interspecific hybridization has also produced other forms with varied foliage colours.

With the exception of genera such as *Cordyline, Phormium,* and *Hebe*, New Zealand native flora has a minimal presence in international markets with only small volumes of other native genera exported as either flowering stems (e.g., *Leptospermum)*, or cut foliage (e.g., *Pittosporum* and *Astelia)*.

Astelia are currently propagated from seed extracted from the berries on female plants, or by plant division, although the latter is relatively slow. Depending on seed age, germination can be erratic. Dividing shoot clumps into single shoots often results in side shoots breaking-off without any stem tissue. Tissue culture offers an alternative means of propagation. There are no published studies on tissue culture of *Astelia* although some commercial laboratories in New Zealand have had limited success with two species. If superior *Astelia* forms, including new hybrid selections, are to be successfully commercialised a rapid clonal propagation system is required. In vitro propagation of New Zealand species has been limited, often undertaken only to conserve the species (Aitken-Christie et al., 1993; Hargreaves et al., 1997). The use of tissue culture to overcome the slowness of conventional propagation methods has had only moderate success (Oliphant, 1993). However, micropropagation techniques have been successfully developed for several native species with ornamental potential (Bicknell et al., 1996; Morgan et al., 1997). This research note reports on progress towards developing an in vitro propagation system for six *Astelia* species with commercial potential.

MATERIALS AND METHODS

Cultures were initiated from either seed or shoot tips. Seeds of *A. nervosa, A. fragrans* (New Zealand Tree Seeds, Rangiora, N.Z.) and *A. chathamica* (Crop & Food Research, Levin, N.Z.) were aseptically placed in culture following sterilisation in a sodium hypochloride solution (1% effective chlorine) for 40 min. It was often necessary to resterilise seeds two or three times during the first 10 days before clean cultures were obtained. Once the seeds were clean, nicking the ends with a scalpel was found to hasten germination. Plants of *A. fragrans, A.banksii, A. nervosa, A. grandis*, and *A. solandri* were obtained from local nurseries. Whole shoots were removed from these plants; where possible, outer leaves were removed and the remaining leaves cut back before sterilising stems as for the seeds. Shoot tips of the terminal shoot (1 to 2 mm in size) and axillary buds positioned in the axils of outer leaves were removed and put into culture.

The pH of all media was adjusted to 5.7 before autoclaving for 20 min at 121C and 103 kPa. The auxin, indoleacetic acid (IAA), when used, was filter sterilised and added to media after autoclaving. All cultures were maintained at 23C with a 16/8 h light/dark photoperiod at a light intensity of 32 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes.

Seeds or shoot explants were placed on media with $^1/_8$ to full strength MS salts (Murashige and Skoog, 1962), LS vitamins, 0 to 0.1 mg $liter^{-1}$ indolebutyric acid (IBA), 0 to 0.3 mg $liter^{-1}$ benzylamino purine (BAP), 30 g $liter^{-1}$ sucrose, and 7.5 g $liter^{-1}$ agar.

To encourage shoot proliferation, individual in vitro shoots of *A. nervosa* were placed on MS media in a factorial experiment with 1.0, 3.0, or 6.0 mg $liter^{-1}$ IBA, IAA, or NAA, and either 3.0 mg $liter^{-1}$ BAP or 3.0 mg $liter^{-1}$ thidiazuron (TDZ). A similar experiment for adventitious shoot initiation compared 0, 0.3, 1.0, and 3.0 mg $liter^{-1}$ NAA with either 0 or 3.0 mg $liter^{-1}$ BAP. Individual shoots were placed on MS media containing 1 to 10 mg $liter^{-1}$ IAA to develop roots before being transferred to the greenhouse where the shoots were planted in a fine pumice mix and placed in a fog tent for 10 days. The shoots were then placed under intermittent mist for a further 2 to 3 weeks.

RESULTS AND DISCUSSION

Within 2 weeks, 60% of fresh seed germinated in vitro whereas seed over 3-months old, even following a 2-month chilling period, took up to 6 weeks to reach fresh seed germination rates. Similar growth rates of seedlings occurred on very dilute MS media ($^1/_8$-strength MS salts) compared to full-strength media. Fewer than 5% of seedlings formed two or more shoots on media with 0.3 mg $liter^{-1}$ BAP. Within a month, shoot tips generally developed into single shoots 1 cm tall. After 2 months in culture, *A. banksii* shoot tips developed clumps of 3 to 5 shoots on MS medium supplemented with 0.05 mg $liter^{-1}$ IBA and 0.3 mg $liter^{-1}$ BAP. These were divided into either single or double shoots and continued to proliferate when placed back onto similar media. While shoot tips of the other species developed into healthy green shoots on this medium, they rarely proliferated additional shoots. *Astelia nervosa* shoots, grown on MS media supplemented with NAA and either 3.0 mg $liter^{-1}$ BAP or TDZ, had smaller leaves and the base of the shoots was considerably swollen, although no additional shoots were visible. This response did not occur when NAA was replaced with either IAA or IBA.

Astelia fragrans shoots grown in the presence of 1.0 or 3.0 mg liter^{-1} NAA and BAP were greener and more vigorous (some had multiple shoots) than shoots grown on similar media with either 0 or 0.3 mg liter^{-1} NAA and BAP. In contrast, *A. nervosa* shoots had more basal swelling when exposed to the higher NAA rates although leaf growth was similar on all media. Minimal proliferation occurred on media with up to 3.0 mg liter^{-1} BAP in the absence of an auxin.

Removing the outer leaves from the swollen basal parts of *A. nervosa* shoots growing on media with NAA, and either BAP or TDZ, revealed meristematic tissue with various degrees of differentiation including distinctive shoot tips. Three to four weeks after placing this new tissue on MS media with 0.3 mg liter^{-1} BAP, clusters of up to 12 adventitious shoots developed with leaves up to 4 cm long.

A two-stage proliferation protocol is therefore being developed. In the first stage, an MS medium supplemented with 1.0 to 3.0 mg liter^{-1} NAA and 3.0 mg liter^{-1} of either BAP or TDZ is used for the initiation of adventitious buds. The second stage, a shoot-elongation medium, has little or no auxin and only low levels of BAP. Long exposure to media with TDZ has been reported to suppress shoot elongation (Seelye and Butcher, 1991). Pulsing tissue on media with TDZ followed by a period on media with no, or minimal, growth regulators has been effective for some shoot regeneration systems (Seelye et al., 1994).

In our studies to date, shoots of *A. nervosa* and *A. banksii* developed roots on an MS medium supplemented with up to 10.0 mg liter^{-1} IAA without loss of shoot quality. Roots also formed on growth-regulator-free media after 6 to 8 weeks. Plants transferred to a fine pumice potting medium and placed under high humidity greenhouse conditions developed a vigorous root system. Three to four weeks after removal from culture, acclimatized plants were being maintained on open benches under normal greenhouse conditions.

SUMMARY

We are successfully developing micropropagation techniques for a number of *Astelia* species which will enhance the commercial development of new selections and hybrids. Our preliminary studies, mainly with *A. nervosa,* found that adventitious buds were initiated on an MS medium with 1.0 to 3.0 mg liter^{-1} NAA plus 3.0 mg liter^{-1} BAP or TDZ. The shoots were then transferred to a shoot-elongation medium with little or no auxin and low levels of BAP. Shoot clusters developed with up to 12 adventitious shoots. Roots developed relatively quickly on a medium containing IAA and these were successfully acclimatized in a greenhouse. Following this promising start, studies are continuing to compare the effect of BAP and TDZ on the initiation of adventitious shoots for the six species of *Astelia*, and the subsequent ability of shoots derived from highly regenerative tissue to initiate and develop a good root system.

LITERATURE CITED

Aitken-Christie, J., K. Gough, L. Holland, S. van der Maas, H. Davies, and **G. Platt.** 1993. Micropropagation of *Agathis australis* (kauri). Abstract NZ IAPTC Meeting 1993. p 27.

Bicknell, R.A., A. Evans, and **E.R. Morgan**. 1996. Micropropagation of *Ranunculus lyalli.* N.Z. J. Crop Hort. Sci. 24:303-306.

Hargreaves, C.L., D.R. Smith, M.N. Foggo, and **M.E. Gordon**. 1997. Propagation and cryopreservation of *Cheesemania* n. sp. "Chalk range"—an endangered New Zealand brassicaceous plant. Abstract N.Z. IAPTC Meeting, p 35.

Metcalf, L. 1993. The cultivation of New Zealand plants. Godwit Press, New Zealand.

Moore, L.B. and **E. Edgar.** 1970. Flora of New Zealand Volume II. Government Printer, Wellington

Morgan, E.R., R.M. Butler, and **R.A. Bicknell**. 1997. In vitro propagation of *Gentiana corymbifera* and *Gentiana cerina*. N.Z. J. Crop Hort. Sci. 25:1-8.

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Oliphant, J.L. 1993. Attempts to micropropagate *Phyllocladus trichomanoides* and *P. glaucus*. (Abstract). N.Z. IAPTC meeting 1993. p. 18.

Seelye, J.F. and **S.M. Butcher**. 1991. In vitro response of *Actinidia* leaf tissue to thidiazuron. N.Z. J. Crop Hort. Sci. 19:447-550.

Seelye, J.F., D.J. Maddocks, G.K. Burge, and **E.R. Morgan.** 1994. Shoot regeneration from leaf discs of *Limonium perigrinum* using thidiazuron. N.Z. J. Crop Hort. Sci. 22:23-29.