Micropropagation of the Pineapple Lily, *Dasypogon hooked* J. Drumm.

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Three genera of arborescent monocots (Staff and Waterhouse, 1981) are native to Western Australia. The genus *Dasypogon* (Dasypogonaceae) is endemic to Western Australia and contains three species. *Dasypogon hookeri*, or pineapple lily, is common in the extreme southwest of Western Australia and is the only arborescent member of the genus, attaining heights of up to 4 m. *Dasypogon hookeri* produces numerous strap-like leaves 30 to 40 cm long and heads of white drumstick flowers in the spring. The height and flowers of *D. hookeri* make it an attractive garden plant (Fig. 1a).

The terminal shoot of *D. hookeri* has not been observed to branch; however, basal clusters of shoots, some produced at the end of 20- to 30-cm-long underground rhizomes, occur after burning of the habitat. Removal or damage to the apical meristem is likely to result in the death of the plant. To date no method other than transplanting whole plants has been successful for propagating this species. This paper details in vitro methods of propagation and establishment in pots of *D. hookeri* using basal shoot clusters as explant sources.

Shoots were excised from clumps of shoots arising from the stem base of a 1-m-tall plant in its natural habitat that had been subjected to a moderate-intensity bush fire 8 months earlier. The tough outer leaves were removed and the shoots washed under running tap water for ≈ 1 h and surface sterilized for 7 min with agitation in 1% w/v sodium hypochlorite with 0.05% w/v Tween-80. Shoots were rinsed in three changes of sterile distilled water, and the outer leaves were carefully removed, taking care not to damage the sheathing leaf base enclosing the apical meristem. These tissues containing the apical meristem were then rinsed for 5 sec in 0.5% sodium hypochlorite only, rinsed thoroughly in sterile distilled water, and dried on sterile filter paper. Twenty-two shoots were placed in 30-ml-capacity polycarbonate tubes (with closures) containing 10 ml of Murashige and Skoog (1962) major and minor mineral salts, supplemented with 100 μ M sodium-ferric EDTA, 500 μ M myo-inositol, 3 μ M thia-

mine HCl, 2.5 μ M pyridoxine HCl, 4 μ M nicotinic acid, 90 mM sucrose, pH 6.0, and 0.1% w/v agar. Media were autoclaved at 121C for 15 min and sloped to cool. Cultures were placed under cool-white fluorescent lamps with light intensity of 40 μ mol-m⁻²-s⁻¹ at culture level and 16/8 h light/dark. About 60% of primary explants were contaminated with bacteria after 14 to 21 days. A further 10% attrition of shoots occurred as a result of vitrification that became evident 2 to 3 weeks after culture.

Four weeks after initiation, sterile shoots were transferred to MS medium containing N- (phenylmethyl)-1 H- purine-6-amine (BA) at 1 and 10 μ M and incubated for 5 weeks. Shoot multiplication was assessed after the fifth and sixth passages on multiplication medium. Each treatment was repeated twice with a total of 25 shoots per treatment, and results were pooled. A 3- to 5-fold increase (mean number of shoots per explant, 3.8 $\pm$ 0.3) per 20-mm-long explant was achieved with 1 μ M A (Fig. 1b). A > 12-fold shoot increase (mean number of shoots per explant, 12.2 $\pm$ 0.6) was achievable with 10 μ M BA. Shoots were stunted on medium with BA at

10 μ M and required an intermediate period of elongation on MS without growth hormones. Elongation of shoots on medium with 1 μ M BA was not required. Also, the incidence of vitrification was more frequent at 10 μ M BA. Previous trials on a related species, *Calectasia grandiflora* Preiss (Xanthorrhoeaceae), indicated that BA was most effective in inducing shoot proliferation compared with other cytokinins (E.B., unpublished).

Root induction was tested in vitro by incubating unrooted shoots on half-strength MS medium supplemented with either 1 H- indole-3-butyric acid (IBA) or α - naphthaleneacetic acid (NAA) at 1, 5, and 10 μ M, but was not successful. We also used nonrooted shoots in vitro that were acclimatized in a shaded area of a greenhouse for 1 week. These shoots were removed from the culture vessels and their bases dusted with softwood rooting powder containing IBA at 0.1% w/w. Incubation time was 5 weeks and a total of 50 shoots were used in two trials. The shoots were then potted into 100-ml black plastic pots containing equal volumes of peat that was pasteurized at 70C for 1 h and unpasteurized perlite. Potted plants were maintained in a constantly fogged glasshouse for 3 weeks, then moved to intermittent misting until rooting was observed. The mean percentage of shoots forming roots after 2 months was 69.8% $\pm$ 8.5%. *Dasypogon hookeri* produces fleshy leaves that appear able to resist desiccation, and shoots are able to remain turgid until roots form. Roots were initially short and thick, i.e., 5 to 10 mm long and 2 to 3 mm in diameter, followed by production of finer lateral roots (Fig. 1c). After 6 months some plants developed additional basal shoots. All plants responded to repotting into 250-ml pots containing a noncommercial potting mixture of equal volume of composted wood fines : coarse sand : perlite, supplemented with inorganic fertilizer and pasteurized at 70C for 1 h. After 2 to 3 months, plants were transferred to routine nursery conditions. At 1 year, plants had grown vigorously (Fig. 1d), producing leaves up to 30 cm long.

This study outlines a basic in vitro propagation procedure using direct in vivo rooting for *D. hookeri*. Additional development of the techniques described (e.g., trying other cutting powders, liquid or gel auxin dips to improve rooting) would expedite commercial propagation of this attractive species.

Literature Cited

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Fig. 1. Plants and shoots of *Dasypogon hookeri*: (a) growing in Jarrah (*Eucalyptus marginata* Donn ex Smith) woodland south of Perth, Western Australia; bar = 25 cm; (b) with basal shoot clusters (left) and elongated basal shoots (right) ready for division for continued culture or potting out as microcuttings, bar = 1 cm; (c) showing root development on a microcutting after 6 weeks, bar = 1 cm; (d) 6 months after removal from culture to potting mix, bar = 3 cm.

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