Micropropagation of *Stirlingia latifolia* (Proteaceae), an Important Cut Flower from Western Australia

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Stirlingia latifolia (R. Br.) Steudel is the most popular wild-picked Proteaceae in Western Australia, accounting for 1.5 million stems annually in 1981 (Burgman and Hopper, 1982). The recalcitrance of blueboy to conventional seed or cutting propagation methods has precluded the plant from commercial horticulture. This paper outlines a method for micropropagation of *S. latifolia* with application for mass production of the species on a commercial scale.

Shoot tips 20 to 30 mm long were taken in midspring (October) from mature, actively growing plants in the field and agitated for 5 min in 0.05% Tween-80 detergent, then surface sterilized in 2% NaOCl for 5 min. The shoots (50) were then trimmed to 10 mm in length, rinsed thoroughly in three changes of sterile distilled water, and placed upright in 30-ml-capacity polycarbonate tubes (with closures) containing 10 ml of half-strength Murashige and Skoog (1962) basal salts with 100 μ M sodium-ferric EDTA, 1 μ M thiamine HCl, 60 mM sucrose, 500 μ M myo-inositol, pH 6.0, and agar at 10 g-liter⁻¹. Media were autoclaved at 121°C for 15 min, sloped to cool, and one explant was placed in each tube. Twenty-four percent of initial explants were sterile and were multiplied on half-strength MS medium supplemented with *N*-(phenylmethyl)-1 *H*-purine-6-amine (BA) or *N*-(3-methyl-2-butenyl)-1 *H*-purine-6-amine (2iP) 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. Shoots were incubated for 4 to 5 weeks and shoot multiplication was assessed after the fourth and fifth successive incubation periods. On medium supplemented with 5 μ M 2iP most explants multiplied by 7-fold, and on medium supplemented with 0.5 μ M BA most explants multiplied by $\geq$ 9-fold (Table 1). BA at 1 μ M induced prolific basal shoot production, but shoots seldom grew longer than 5

to 7 mm and at least one-fourth of the shoots were vitrified. Although fewer shoots were produced from explants incubated on media supplemented with 2iP, these shoots grew much longer, i.e., 15 to 30 mm, with little or no incidence of vitrification.

In one trial, stunted basal shoots were excised from shoot clumps after 3 to 4 weeks of incubation on multiplication medium with 0.5 μ M BA. Twenty-five shoots were placed on half-strength MS medium without plant growth regulators but supplemented with 0.04% w/v activated, neutralized charcoal. Twenty-one shoots were placed on half-strength MS medium supplemented with 1 μ M of gibberellic acid (GA₃). Both media suppressed further shoot multiplication and allowed shoots to elongate from two to four times their original length; however, medium supplemented with charcoal resulted in green robust shoot development compared with the yellowish-green, thin shoots produced in the presence of GA₃.

Shoots 15 to 30 mm long were tested for root initiation on half-strength MS medium supplemented with auxin (1, 5, and 10 μ M IBA) and as microcuttings using softwood cutting powder (with 0.01% w/w available IBA) applied to the base of shoots. Rooted and nonrooted shoots were acclimatized before removal from culture by transferring culture vessels to the glasshouse with 70% shade for several days. An initial potting mixture drench with 0.15% aqueous propyl[3-(dimethylamino) propyl] carbamate (propamocarb) antifungal solution was used. Microcuttings were placed initially in trays

Table 2. Percentage of *Stirlingia latifolia* shoots producing roots in vitro and in vivo, in response to auxin*.

Growth regulators (μ M)	Mean rooting (%) $\pm$ SE
<i>In vitro</i>	
IBA	
0	0
1.0	14.5 $\pm$ 4.5
5.0	27.5 $\pm$ 12.5
10.0	55.5 $\pm$ 12.5
<i>In vivo</i>	
IBA (0.01% w/w) in cutting powder	80.0 $\pm$ 10.0

*Per 7-week incubation. Treatments replicated 20 times each, repeated twice, and results pooled.

of pots of 40-ml capacity (64 per tray) and grown in constantly fogged conditions until roots were formed, then moved to intermittent misting. In vitro rooting (Table 2) was not satisfactory; roots tended to grow from basal callus. Such shoots performed poorly when potted, possibly as a result of incomplete vascular connections and some unavoidable damage to roots on removal from agar and during transfer to potting mixture. In contrast, >90% of unrooted shoots survived the transfer to pasteurized peat : perlite (v/v) potting mixture, and 80% of these shoots produced roots after 7 weeks (Table 2). Growth of microcuttings was healthy with plants 20 to 30 cm high 9 months after transplanting from culture and flowering within a further 3 months under standard nursery conditions.

Micropropagation of *S. latifolia* should help limit the undesirable practice of bush picking and result in improvements in the quality and availability of this important species for commercial horticulture production.

Literature Cited

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Table 1. Shoot production per explant of *Stirlingia latifolia* placed on half-strength MS medium supplemented with 2iP or BA*.

Growth regulators (μ M)	Frequency of shoots/explant ^y				Mean no. shoots/explant $\pm$ SE
	1-3	4-6	7-9	>9	
2iP					
0	100	0	0	0	1.1 $\pm$ 0.1
2.5	50	45	5	0	3.6 $\pm$ 0.3
5.0	0	35	65	0	6.9 $\pm$ 0.3
BA					
0.5	0	5	40	55	10.2 $\pm$ 0.5
1.0	0	0	0	100	13.9 $\pm$ 0.5

*Per 5-week incubation period. Treatments replicated 20 times each, repeated twice, and results pooled.

^yPercent of total in each category.

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