Micropropagation of *Boronia*

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Successful micropropagation was achieved in four species of *Boronia: B. edwardsii* (Benth.), *B. filifolia* (Benth.), *B. pilosa* (Labill.) and *B. ruppii* (Cheel.). Shoot tips or nodal explants were initiated on half-strength Murashige and Skoog (MS) basal medium. A five-fold multiplication rate occurred every 4 weeks on basal medium supplemented with 1 mM each of 6-benzylaminopurine (BAP) and 6-furfurylaminopurine (Kin or Kinetin). All species produced roots on MS basal medium supplemented with various auxins. More than 90% of the rooted plantlets became acclimatized and flowered within 6 months from the date of transfer to the glasshouse.

Chemical names used: 6-furfurylaminopurine (kinetin); 6-benzylaminopurine (BAP); 1-H-indole-3-butyric acid (IBA); α-naphthaleneacetic acid (NAA); α-naphthoxyacetic acid (NOA).

INTRODUCTION

The genus *Boronia* (Rutaceae) contains about 95 species, all of which are endemic to Australia. Most species of *Boronia* occur in heath and dry sclerophyll forests where vegetation is thick and shade is available. They are mostly small, woody shrubs of 1 m or less with flower colour in varying shades of pink, mauve, red, yellow or brown. Flowers have four petals, opening widely like a star in some species (e.g., *B. edwardsii),* but remaining concave and cup-like in others (e.g., *B. megastigma)*. Most have aromatic foliage and in some the flowers are highly perfumed. This genus includes some of the most popular ornamental plants in the Australian flora.

Tissue culture is one means of producing large quantities of uniform plants irrespective of season and climatic conditions. Our objective was to develop micropropagation protocols for 4 species of *Boronia*: *B. edwardsii* (Benth.), *B. filifolia* (Benth.), *B. pilosa* (Labill.), and *B. ruppii* (Cheel.). All are listed as threatened Australian species (Leigh et al., 1981).

EXPERIMENTAL PROCEDURES, RESULTS AND DISCUSSION

As the success of any tissue culture project depends upon a supply of healthy stock plants (Taji et al., 1992), cutting material was collected from field-grown plants. Cuttings were treated for 5 sec with a range of auxins in 50% ethanol (Table 1). Rooted cuttings were grown in a pasteurized mixture of 2 sand : 1 peat : 1 perlite

(by volume) with 4 g/litre of Osmocote® slow release fertilizer (16N : 4.4P : 8.3K) in pots 15 cm in diameter. Resulting plants were kept in a dry glasshouse (25C day temperature and 15C night temperature) with no overhead watering and sprayed weekly with a 0.1% (w/v) Benlate solution (50% active benomyl). Pot-grown plants were 6 months old when shoots were taken for *in vitro* studies. Shoot tips 4 to 7 cm long were taken from recent growth flushes and washed under running tap water for 2 h. They were then surface sterilized by successive immersion in 70% ethanol for 30 sec; 0.5% (w/v) sodium hypochlorite containing 0.01% Triton (octyl phenoxy polyethoxy ethanol, a wetting agent) for 15 min, followed by two rinses in sterile water. Nodal and apical segments 0.5 cm long were placed in sterile medium. All media used included half-strength Murashige and Skoog's (1962) basal medium with 60 mM sucrose and 8 g/litre Difco BiTek™ agar. The pH was adjusted to 5.5 using 1 N NaOH or HCl before autoclaving at 121C and 103 kPa for 15 min. Ten ml of medium were used per 8 × 2.5-cm screw-capped polycarbonate tube. All cultures were incubated at 25C under cool-white fluorescent light with an irradiance of 50 mmol m^{-2} s^{-1} and a 16 h photoperiod.

Table 1. Effect of auxin concentration on the rooting response of shoot cuttings of four *Boronia* species to auxin[1].

	% of rooted cuttings					
	B. edwardsii		*B. filifolia*		*B. pilosa*	*B. ruppii*
Auxins (ppm)	Late Summer	Winter	Mid Summer	Early Spring	Mid Summer	Summer
Control	0	0	0	0	0	0
1000 NAA	75	30	0	0	0	15
500 IBA + 500 NAA	60	23	0	28	0	20
500 IBA + 500 NOA	63	28	0	36	10	25
1500 IBA + 500 NAA	81	40	0	26	0	10

[1] 100 cuttings per treatment.

For all species under investigation, the best shoot proliferation was achieved on half-strength MS medium supplemented with 1 mM each of BAP and Kin (Table 2). After five subcultures the shoots were transferred to basal medium (without added hormones) for 5 weeks to allow shoot elongation. Microcuttings 3 to 5 cm long were placed in basal medium containing auxins, as per Table 3, for root initiation.

Boronia species had specific auxin requirements for root formation. The highest percentage of cultures to produce roots was achieved on 2 mM IBA for *B. edwardsii* (65%) and *B. pilosa* (50%), 1 mM IBA + 1 mM NOA for *B. filifolia* (41%), and 1 mM IBA + 1 mM NAA for *B. ruppii* (20%) (Table 3).

Rooted explants were transferred to a pasteurized potting mixture containing 1 peat : 1 perlite : 1 sand (by volume) and adapted to glasshouse conditions under 30% daylight at 25C with gradual exposure to reduced relative humidity by gradually removing the glass cover over a period of 2 to 3 weeks. On transfer to a glasshouse

plants were given intermittent misting for 1 week then watered daily with weak soluble fertilizer (N:P:K, 23:4:18; 25 g litre^{-1}). More than 90% of the rooted microcuttings survived the acclimatization procedure and produced flowers within 6 months after transfer.

Table 2. Cytokinin effect on *in vitro* shoot multiplication of four *Boronia* species cultured on half-strength MS[1].

Cytokinin	*B. edwardsii*	*B. filifolia*	*B pilosa*	*B. ruppii*
	Mean shoot number [2]			
0 µM BAP + 0 µM Kin	1.2±0.8	1.5±1.1	1.2±1.0	1.3±1.3
0.1 µM BAP + 0.1 µM Kin	2.0±1.5	2.6±1.6	2.5±2.3	2.1±1.3
1.0 µM BAP + 1.0 µM Kin	5.6±0.8	5.5±1.1	5.4±1.3	4.9±1.0
10 µM BAP + 10 µM Kin	x[3]	x[3]	x[3]	x[3]

[1] Subcultured every week.
[2] Means of 3 experiments each with 5 replicates, standard error.
[3] In all the species, dense clumps of very short shoots were produced in which it was difficult to count shoot numbers.

Table 3. Auxin effect on *in vitro* rooting of *Boronia* cultured on half-strength MS after four weeks.

Auxin	*B. edwardsii*	*B. filifolia*	*B. pilosa*	*B. ruppii*
	% of Cultures with roots[1]			
0	0	0	0	0
2 µM IBA	65±21	0	50±10	0
1 µM IBA + 1 µM NAA	15±7	37±14	0	20±7
1 µM IBA + 1 µM NOA	10±0	41±17	0	0

[1] Means of three experiments each with 15 replicates, standard error.

CONCLUSION

This paper outlines methods for rapid propagation of four species of *Boronia*. Work in progress is aimed at improving the percentage of rooting of these species of *Boronia*, particularly *B. ruppii*. In vivo rooting is also under study which could expedite commercial propagation of these attractive species.

LITERATURE CITED

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