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ADVENTITIOUS BUD FORMATION AND REGENERATION IN TISSUE CULTURES OF BRACHYCOME IBERIDIFOLIA BENTH.

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SUMMARY

Leaf, stem and cotyledon explants of Swan River daisy (*Brachycome iberidifolia* Benth.) exhibit organogenic capacity when placed on nutrient media containing auxin and cytokinin. Maximum production of adventitious shoots occurred when leaf material was cultured on a medium containing 10 μM benzylaminopurine (BAP) and 5 nM napthaleneacetic acid (NAA). Elongated adventitious shoots were readily rooted on a medium containing 5 nM indole-3-acetic acid (IAA) alone.

1. INTRODUCTION

The morphogenetic potential and phenotypic and chromosomal stability of cultures of *Brachycome dichromosomatica* have been reported (GOULD 1978, 1979). The present brief paper defines the cultural parameters and the interactions of auxins and cytokinins affecting production of adventitious shoots in cultured tissues of *B. iberidifolia* and presents a protocol for plantlet regeneration.

2. MATERIALS AND METHODS

Seeds of *Bracycome iberidifolia* Benth. (Asteraceae) were obtained from the J. L. Hudson Seed Co., Redwood City, CA USA. Seeds were surfaced sterilized (0.525% sodium hypochlorite for 10 min) and germinated on a nutrient medium (KIRBY & CHENG 1979) at pH 5.5 containing 3.0% sucrose and 0.6% Bacto-Agar (Difco Laboratories, Detroit, MI USA). In order to investigate the ability of cultured explants of *B. iberidifolia* to produce adventitious shoots, leaf, stem and cotyledon explants from 2–3 week seedlings were cultured on the medium described containing auxin (indole-3-acetic acid [IAA]; α-naphthaleneacetic acid [NAA]) and cytokinin (N_6-benzylamino purine; N_6-(Δ^2-isopentenyl) adenine [2iP] (Sigma Chemical Co., St. Louis, MO USA). All cultures were maintained in a constant temperature facility (24 °C ± 0.5 °C) with a photoperiod of 18 h (180 $\mu E\ m^{-2}$) followed by 6 h darkness.

3. RESULTS AND DISCUSSION

Results of experiments assessing the ability of stem, leaf and cotyledon explants

Table 1. Effects of IAA and 2iP on Adventitious Shoot Formation in Leaf and Cotyledon Cultures of *Brachycome iberidifolia*.

IAA Level	2iP Level	percentage of cultures forming adventitious shoots	
		Leaf	Cotyledon
5 nM	1 μM	83	0
5 nM	5 μM	70	25
5 nM	10 μM	45	20
5 nM	20 μM	30	15
50 nM	1 μM	63	15
50 nM	5 μM	45	37
50 nM	10 μM	25	25
50 nM	20 μM	30	15

Leaf and cotyledon material was isolated from 10 day old aseptically grown seedlings. Observations were made on 30 cultures of 4 explants each from each of 2 experiments after 21–28 days in culture.

Table 2. Effects of NAA and BAP on Adventitious Shoot Formation in Stem and Leaf Cultures of *Brachycome iberidifolia*.

NAA Level	BAP Level	percentage of cultures forming adventitious shoots	
		Leaf	Stem
5 nM	0.1 μM	62	18
5 nM	1.0 μM	90	10
5 nM	10.0 μM	94	0
50 nM	0.1 μM	44	12
50 nM	1.0 μM	68	28
50 nM	10.0 μM	93	4

Stem and cotyledon material was isolated from 10 day old aseptically grown seedlings. Observations were made on 30 cultures of 4 explants each from of 2 experiments 21–28 days in culture.

to form adventitious shoots are presented in *tables 1* and *2*. Explants of cotyledons, stems and leaves readily produce adventitious shoots in culture. Leaf explants from multiple buds in up to 94% of cultures when placed on a medium supplemented with 10 μM BAP and 5 nM NAA (*fig. 1A*). Under such conditions, buds are first observed as early as 5 days after leaf explants are placed in culture. Cotyledon and stem explants (*figs. 1B* and *1C*) are also capable of producing adventitious buds, but at levels considerably below that achieved with leaf explants (37% and 28%, respectively). The ability of leaf cultures of exhibit higher rates of organogenesis may, in part, be related to endogenous levels of plant growth regulators, particularly cytokinins. Interestingly, 2iP, a naturally-occurring cytokinin, is capable of eliciting maximum bud formation at relatively low levels 1 μM) when compared with BAP, a synthetic cytokinin, which elicits maximum production of adventitious buds at a ten-fold higher concentration.

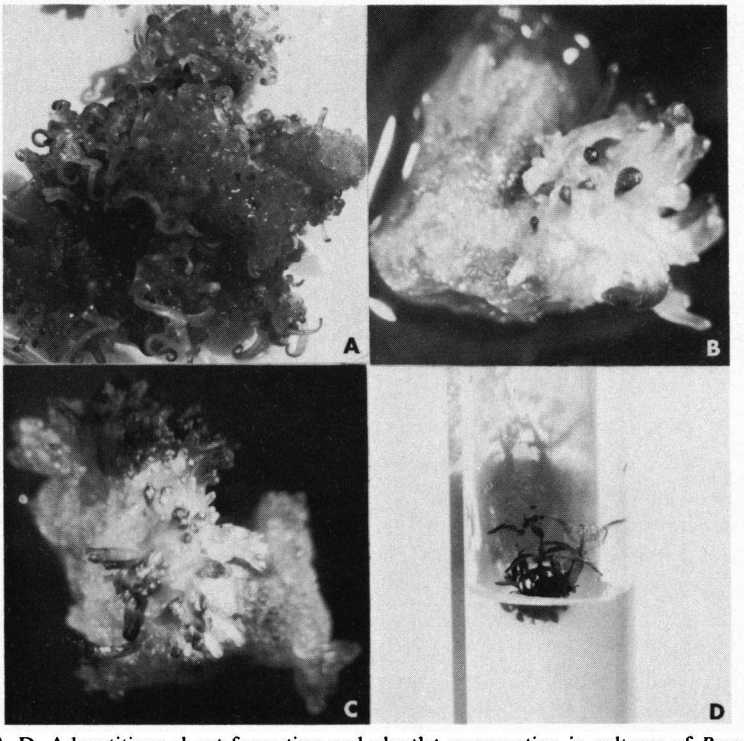

Fig. 1 A–D. Adventitious shoot formation and plantlet regeneration in cultures of *Brachycome iberidifolia* Benth. A. Leaf culture after 28 days on medium containing 10 μM BAP and 5 nM NAA ($\times$6); B. Cotyledon culture after 21 days on medium containing 10 μM 2iP and 50 nM IAA ($\times$7); C. Stem culture after 21 days on medium containing 10 μM BAP and 50 nM NAA ($\times$7); D. Early stages in the rooting of adventitiously produced shoots on medium containing solely 5 nM IAA ($\times$0.5).

Buds produced from leaf, stem and cotyledon cultures of *B. iberidifolia*, can readily produce plantlets by placing elongated shoots on an agar medium supplemented with 5 nM IAA in the absence of cytokinin (*fig. 1D*). The ease with which this species may be manipulated *in vitro* coupled with its low chromosome number (2n = 8) and the apparent genetic stability of the genus *in vitro* (GOULD, 1979), indicates that *Brachycome iberidifolia* may prove a useful plant for future genetic and physiological research.

REFERENCES

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