Plant Regeneration in *Coreopsis lanceolata* L. from Leaf Tissue Cultures

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Additional index words. tissue culture, shoot differentiation, organogenesis, lance coreopsis

Abstract. Excised leaf sections of lance coreopsis cultured on Murashige Skoog (MS) medium produced adventitious shoots in response to BA. When the combinations of 0, 0.5, 1, or 2 μM NAA with 0, 5, 10, 20, or 40 μM BA were tested, shoots were induced by any of the four BA concentrations used in the medium, regardless of the presence of NAA. The average number of shoots formed per leaf section ranged from 1.4 to 4.3 seven weeks after culture initiation. Roots were induced at the base of individual shoots on the same regeneration medium when cultures were kept longer than 7 weeks. The rooted plants were transferred successfully into soil. The regenerated plants had the same growth and flowering characteristics as the seed-grown plants. Chemical names used: benzyladenine (BA); naphthaleneacetic acid (NAA).

Lance coreopsis (Asteraceae) is a yellow, flowering perennial commonly grown as a garden plant. It is well adapted to a wide range of climatic conditions from the southern to the northern central parts of the United States. The plant is usually propagated by seed or by crown division. Improvement of this ornamental for shorter plant heights, various flower colors and sizes, and early flowering may be possible by selection and genetic manipulation. To our knowledge, tissue culture propagation and genetic studies in this species have not been reported. The objective of this study was to develop procedures for plant regeneration of lance coreopsis via leaf tissue culture.

Materials and Methods

Seeds collected from garden-grown plants of lance coreopsis (supplied by Bluebird Nursery, Clarkson, Neb.) were sown in peat-lite mix (Sunshine Mix #3; Sun Gro Horticulture, Bellevue, Wash.) in bedding plant trays and germinated in a greenhouse. Seedlings at the two true-leaf stage were transplanted into another commercial peat-lite mix (Sunshine Mix #1) and grown in a greenhouse for 5 months. Fully expanded leaves of these greenhouse-grown plants were excised and disinfested for 10 min in 10% household bleach (Clorox, 0.525% sodium hypochlorite) containing 5 μg Tween 20/liter, followed by four rinses in sterile distilled water. Leaf blades were then cut into 8 $\times$ 8-mm pieces under a sterile

laminar flow hood and cultured in 9-cm-diameter plastic petri dishes on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing MS vitamins and

solidified with 7 g Difco Bacto agar/liter.

To study the effects of growth regulators, all combinations of 0, 0.5, 1, or 2 μM NAA with 0, 5, 10, or 20 μM BA were used in the culture medium for the first experiment. A second experiment was carried out with the same NAA concentrations combined with 0, 10, 20, or 40 μM BA. For both experiments, each treatment consisted of nine leaf sections in a petri dish. Five replications per treatment were used. A total of 720 leaf sections (nine disks per treatment $\times$ five replications $\times$ 16 treatments) was used for each experiment. The petri dishes were randomly assigned and incubated for 7 weeks on a culture rack under cool-white fluorescent lamps that provided a photosynthetic photon flux of 80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a 16-h photoperiod. The culture room was maintained at $22 \pm 2^\circ\text{C}$ and 40% to 60% relative humidity. Treatment effects were evaluated for percentage of leaf sections forming shoots and the average number of shoots and roots that had been produced per explant 7 weeks after culture initiation. Data on the percentage of cultures forming shoots and roots were analyzed after arcsin transformation and then converted back to original values for inclusion in tables. Statistical analysis was performed using the step-wise regression model in the CoStat program (CoHort Software, Minneapolis).

Fig. 1. Plant regeneration from *Coreopsis lanceolata* leaf tissues: (A) leaf section explants beginning to swell after 2 weeks in culture (left), and shoot differentiation from leaf sections cultured on MS medium containing 1 μM NAA plus 10 μM BA (right); (B) a 10-month-old regenerated plant established in a 3.5-liter pot containing peat-lite mix.

Received for publication 1 Aug. 1994. Accepted for publication 19 Aug. 1994. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Results and Discussion

Leaf sections began to swell after 2 weeks of culture in all media. Adventitious shoot buds forming on the edges of swollen tissue sections became visible 3 weeks after culture initiation on culture media containing any amount of BA (Fig. 1A). In the first experiment, 16% to 51% of leaf sections formed shoots, depending on BA concentrations (Table 1). When BA was absent in the medium, shoot formation was either completely inhibited (at the 0- or 0.5- μM NAA level) or extremely low (only 2.2% at the 1- or 2- μM NAA level). Although percent shoot formation slightly increased with an increase in BA concentration from 5 to 20 μM , it was difficult to single out the best NAA and BA combination to be used in regeneration medium.

In the second experiment, the range of BA concentrations used was expanded to include 40 μM to find the upper limits of the cytokinin influence. However, data obtained were similar to those of the first experiment. Shoot differentiation was slightly reduced when the medium contained 40 μM BA compared to 10–20 μM BA as indicated by the quadratic, rather than linear, responses of shoot differentiation to BA concentration (Table 2). When the culture medium did not contain BA, shoot formation was completely inhibited, regardless of the presence of NAA. The percentages of cultures forming shoots on medium containing 10 μM BA were similar to those obtained with 20 μM BA. The average number of shoots that developed per culture was also similar to those obtained from Expt. 1.

Individual shoots, when divided and subcultured on the regeneration medium (MS medium containing 1 μM NAA and 10 μM BA), continued to proliferate axillary shoots. The newly formed shoots began to produce roots at the base on the same culture medium after 7 weeks. All rooted plants, when transferred to a peat-lite medium (Sunshine Mix #1) in the greenhouse, survived. The growth and flowering of regenerated plants were similar to those of seed-propagated plants (Fig. 1B).

The results of this experiment indicate that lance coreopsis can be regenerated by culturing leaf sections in vitro. Culture media containing 10–20 μM BA and 1 μM NAA appear to be most favorable for inducing adventitious shoot formation on leaf tissue explants. In general, the number of shoots generated per leaf section was minimal, compared to that obtained from leaf tissue cultures of other plants (Bajaj, 1972; Kothari and Chandra, 1984; Lee et al., 1977; Peck and Cumming, 1984; Smith and Norris, 1983). The plant regeneration system may well be used in future genetic enhancement programs involving the native coreopsis collections.

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Table 1. Shoot and root organogenesis from leaf tissues of lance coreopsis cultured on MS medium supplemented with NAA and BA (Expt. 1).

Growth regulator (μM)		Disks with shoots	No. shoots/ explant ^y	Explants with roots
NAA	BA	(%) ^z		(%) ^z
0	0	0.0	0.0	0.0
0	5	24.4	3.2	0.0
0	10	30.5	2.3	6.7
0	20	44.4	2.1	0.0
0.5	0	0.0	0.0	0.0
0.5	5	27.8	2.5	0.0
0.5	10	24.4	3.2	15.5
0.5	20	15.6	1.4	17.8
1	0	2.2	0.2	0.0
1	5	35.5	3.0	8.9
1	10	42.2	4.3	11.1
1	20	51.1	2.6	8.9
2	0	2.2	0.4	4.4
2	5	29.9	1.6	11.1
2	10	36.1	2.8	11.1
2	20	41.5	3.4	8.3
Significance				
BA concn				
Linear		***	***	NS
Quadratic		***	***	NS
NAA concn				
Linear		NS	NS	NS
Quadratic		NS	NS	NS

^zAverages of 45 leaf sections (nine sections per treatment $\times$ five replications) determined 50 days after culture initiation.

^yAverages taken from leaf sections producing shoots.

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

Table 2. Shoot and root organogenesis from leaf tissues of lance coreopsis cultured on MS medium supplemented with NAA and BA (Expt. 2).

Growth regulator (μM)		Disks with shoots	No. shoots/ explant ^y	Explants with roots
NAA	BA	(%) ^z		(%) ^z
0	0	0.0	0.0	0.0
0	10	33.3	1.2	0.0
0	20	33.3	1.9	8.9
0	40	22.2	1.1	0.0
0.5	0	0.0	0.0	0.0
0.5	10	11.1	1.4	0.0
0.5	20	36.1	2.4	5.6
0.5	40	6.7	1.1	0.0
1	0	0.0	0.0	0.0
1	10	70.7	3.2	15.6
1	20	55.6	1.2	6.7
1	40	20.0	2.2	6.7
2	0	0.0	0.0	4.4
2	10	22.2	2.3	0.0
2	20	20.0	1.4	2.2
2	40	2.2	4.0	0.0
Significance				
BA concn				
Linear		NS	NS	NS
Quadratic		***	***	NS
NAA concn				
Linear		NS	NS	NS
Quadratic		NS	NS	NS

^zAverages of 45 leaf sections (nine sections per treatment $\times$ five replications) determined 50 days after culture initiation.

^yAverages taken from leaf sections producing shoots.

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

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