HortScience 26(12):1555-1557. 1991.

In Vitro and Macropropagation of the Wildflower *Dyssodia pentacheta* (D. C.) Robins

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Additional index words. rooting, shoot proliferation, xeriscape

Abstract. Dyssodia pentacheta, **a prostrate-growing perennial Texas wildflower with potential for use in low-maintenance landscapes, was propagated in vitro and by stem cuttings under mist. Optimum rooting for IBA-treated semihardwood terminal stem cuttings (3 to 30 mM IBA) and in vitro-grown nodal segments (30 to 100 mM IBA) occurred after 4 weeks under an intermittent mist system. A 300-mM IBA basal dip was lethal to macro- and microcuttings. In vitro, *D. pentacheta* produced more shoots per nodal explant on Woody Plant Medium (2 g Gelrite/liter) with 1 to 10 µM BA than with combinations of BA and 0.5 µM NAA. After shoot proliferation, the shoots were subculture twice and grown on growth regulator-free medium. When maintaining *D. pentacheta* in vitro on media devoid of plant growth regulators, 1% sucrose was more effective than 2% for promoting shoot growth and suppressing apparent production of phenolics. Chemical names used: N-(phenylmethyl) -1H-purin-6-amine (BA); 1H-indole-3-butyric acid (IBA); 1-naphthaleneacetic acid (NAA).**

Dyssodia pentacheta is a low-growing, perennial wildflower of the southwestern United States with small (1 cm), prolific, yellow composite flowers and evergreen foliage (Campbell and Loughmiller, 1985; Correll and Johnston, 1979). It thrives in arid environments with calcareous soils (pH 7.5-8.5) and has potential for use in xeriscapes (Enquist, 1987). Although no research has been reported on the propagation of this genus, the related genera *Tagetes* and *Helianthus* have been propagated in vitro (Binding et al., 1981; Kothari and Chandra, 1984). The goals of this research were to develop propagation procedures for shoot proliferation in vitro and rooting of micro- and macrocuttings with intermittent mist.

Semihardwood terminal stem cuttings (3 to 5 cm) were taken from stock plants grown in a glasshouse [15/35C, average minimum night/maximum day; maximum photosynthetic photon flux (PPF) of 1260 $\mu mol \cdot m^{-2} \cdot s^{-1}$]. Leaves were stripped from the basal 2 cm of each cutting. The bases of the cuttings were dipped for 30 sec in 0, 3, 10, 30, 100, or 300 mM IBA solutions. Cuttings were stuck into flats (3 liters) containing a medium of 1 vermiculite : 1 perlite (v/v). Five flats (replications) of 36 cuttings

Received for publication 13 Nov. 1990. Technical Article no. TA 25932 of the Texas Agricultural Experiment Station. 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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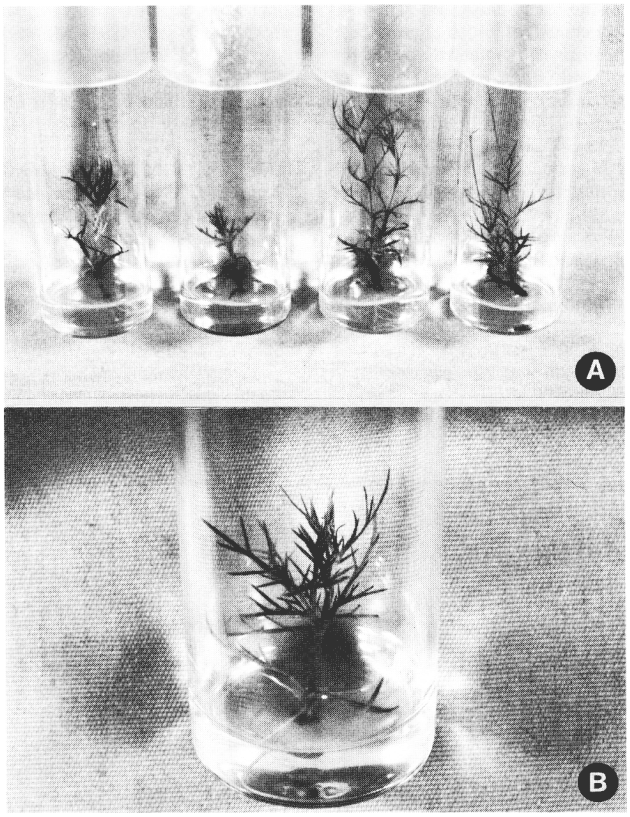

Fig. 1. *Dyssodia pentacheta* shoot development. **(A)** The two tubes to the left contain 20 g of sucrose and 2 and 3 g Gelrite/liter, respectively, the two tubes to the right contain 10 g of sucrose and 2 and 3 g Gelrite/liter, respectively. On media devoid of plant growth regulators, the lower sucrose concentration promoted development of shoots from axillary buds of nodal segments. (B) Terminal shoot explants *of Dyssodia* at 3 weeks during stage L

were prepared and each flat had six samples from each treatment (n = 30). The flats were placed under an intermittent mist system (6 sec every 6 min from 07:00 to 19:30 **HR).** After 4 weeks, the cuttings were harvested and the roots washed. Data collection included percent rooting, the number of roots per cutting, and root length of the three longest roots of each cutting.

Semihardwood terminal cuttings (4 cm long) from shadehouse-grown stock plants were used as explants. To control surface contaminants, explants were surface-disinfected by prerinsing for 1 h in running water, followed by a l-rein 70% ethanol soak, 20 min in 2.62% NaOCl (50% bleach), and three rinses in sterile distilled water. Following this surface disinfection, explants were cut into l-cm nodal segments and placed vertically in 38-cm^3 shell vials containing 6 ml of sterile Woody Plant Medium (WPM) (Lloyd and McCown, 1980),20 g sucrose/liter, and gelled with 2 g Gelrite/liter (Kelco Div., Merck & Co., San Diego) at pH 5.7. All cultures were grown at 26 ± 0.5C under a 16-h photoperiod provided by cool-white fluorescent lights (PPF of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

After 3 weeks, noncontaminated plant material (Fig. lB) was transferred for shoot proliferation (stage II) to WPM supplemented with 0 or 0.5 µM NAA in combination with 0, 1, 3, or 10 µMBA and grown in the above environment. This factorial experiment was a completely randomized design with 26 replications per treatment. Four weeks later, the explants were evaluated for multiple shoot development.

The following treatments were devised to determine the best preacclimatization conditions for in vitro plants (stage III). Shoots were divided and placed vertically into Magenta GA-7 vessels (Magenta Corp., Chicago) on 30 ml WPM without plant growth regulators and grown in the environment noted. After 6 weeks, nodal segments from the in vitro-developed plants were transferred to GA-7 vessels with 30 ml WPM containing 10 or 20 g sucrose/liter in combination with 2 or 3 g Gelrite/liter. Growth and development were evaluated after 6 weeks.

Cuttings with three nodes were taken from 6-week-old preacclimatized in vitro plants, and the basal leaves were removed and dipped for 30 sec in 0 to 300 mM IBA. The cuttings were stuck in flats, placed under intermittent mist as stated, and harvested after 4 weeks. There were four flats (replications) of 60 cuttings, and each flat had 10 samples from each treatment (n = 40). The data from both experiments were tested by either multiple regression or analysis of variance. Treatment means involving percentage data were arcsin-transforrned before analyses.

Table 1. Effect of IBA concentration on rooting of *Dyssodia pentacheta* semihardwood terminal cuttings after 4 weeks under intermittent mist.[z]

IBA[y] (mM)	Rooting (%)	Mean root no.
0	97	5.5
3	100	12.6
10	97	16.0
30	83	15.1
100	70	8.4
300	0	0.0
Significance[x]	Q[w]	LQ

[z]Data are means of 30 observations.
Thirty-sec dip.
[x]Q, LQ = quadratic and linear and quadratic regression significance at $P = 0.05$, respectively.
[w]Regression performed on arcsin-transformed rooting percentages.

The basal leaves on semihardwood terminal stem cuttings turned brown in the 300-mM IBA treatment after 1 week under an intermittent mist system. After 2 weeks, nearly all cuttings from the 300-mM IBA treatment were brown and necrotic. Optimum rooting occurred with a 30-sec dip in 3 to 30 mM IBA (Table 1), but there was no difference in root length (range 2.7-7.0 cm for those that grew). Rooting decreased as the IBA concentration was increased above 30 mM. Roots formed along the length of the stem treated with 30 mM and lower IBA solutions. The area of stem above the treated area formed roots with 100 mM IBA. With 300 mM IBA, roots only formed from the base of a few leaves that were no longer attached to the stem. *Dyssodia pentacheta* appears to be similar to *Tagetes patula* L., which had a high percentage of cuttings that developed adventitious roots without exposure to exogenous auxin (Kothari and Chandra, 1982, 1984).

In vitro shoot proliferation from axillary buds was higher when BA was used alone in the medium rather than in combination with NAA (Table 2). However, the average shoot count varied little among the 1 to 10-µM BA treatments. More numerous but shorter shoots were produced with 10 µM BA than with the other concentrations; however, since only shoots ≥ 5 mm long were recorded, shoot count was similar among the BA treatments.

The reduction in shoot proliferation and development when NAA was used in combination with BA was contrary to observations with *T. patula* by Kothari and Chandra (1984). The presence of NAA in the medium caused excessive callus formation at the base of D. *pentacheta* nodal explants. The proliferation of callus indicates that the auxin concentration may have been too high, possibly causing a competing sink-to-shoot proliferation. The presence of phenolics was evident by the browning of the callus and medium.

On media devoid of plant growth regulators in GA-7 vessels, sucrose at 10 $g \cdot liter^{-1}$ promoted the development of shoots from axillary buds of nodal segments, whereas the normal WPM sucrose level (20 $g \cdot liter^{-1}$) suppressed development (Table 3 and Fig. 1A). The nodal segments without axillary

Table 2. Shoot proliferation of *Dyssodia pentacheta* after 33 days in vitro.

Plant growth regulators (μM)		Mean no. shoots/explant[z]
BA	NAA	
0	0	1.2
1	0	6.3
3	0	7.2
10	0	6.6
0	0.5	2.9
1	0.5	2.8
3	0.5	4.7
10	0.5	4.5
Significance		
BA		*
NAA		*
BA × NAA		NS

[z]Data are means of 26 observations.
[NS,*]Nonsignificant or significant at $P = 0.05$, respectively.

Table 3. Effect of sucrose and Gelrite concentrations on the growth of *Dyssodia pentacheta* on media devoid of plant growth regulators.

Sucrose (g·liter^{-1})	Gelrite (g·liter^{-1})	Growth from axillary bud (%)	No. shoot growth (%)
10	2	71.6[z]	7.4
	3	59.2	3.7
20	2	24.6	29.6
	3	28.3	34.5
Significance			
Sucrose (Suc)		***	***
Gelrite (Gel)		NS	NS
Suc × Gel		NS	NS

[z]Data are mean percentages of 81 samples and percentages were arcsin-transformed before analysis.
[NS,***]Nonsignificant or significant at $P = 0.001$, respectively.

Table 4. Rooting of *Dyssodia pentacheta* cuttings from in vitro-grown shoots after 4 weeks under intermittent mist.[z]

IBA[y] (mM)	Rooting (%)	Mean root no.
0	83	4.2
3	95	5.3
10	93	5.4
30	88	7.8
100	95	6.9
300	0	0.0
Significance[x]	Q[w]	Q

[z]Data are means of 40 observations.
[y]Thirty-sec dip.
[x]Q = quadratic regression significance at $P = 0.05$.
[w]Regression performed on arcsin-transformed rooting percentages.

shoot growth remained green and without callus formation or the presence of phenolies. The low sucrose/low Gelrite combination resulted in the fewest nodal segments that, presumably, accumulated phenolic compounds and died (range 21% to 46%), but the differences were nonsignificant.

In vitro-grown nodal segments rooted well when removed from the culture environment and placed under an intermittent mist system. A high percentage of rooting for all IBA treatments was observed, except for the 300-mM level, which was again lethal to the nodal segments (Table 4). The 30- and 100-mM IBA levels resulted in more (Table 4), but not longer (range 3.5-4.2 cm) roots per cutting than 0 to 10 mM IBA.

Plants grown in vitro produced fewer and shorter roots than semihardwood terminal cuttings (Table 1). One possible explanation for this difference is daylength. Semihardwood terminal cuttings were stuck in late September and the in vitro cuttings were stuck in mid-November, which resulted in a subsequent 2-h reduction in photoperiod and a decrease in maximum greenhouse temperature.

In summary, a system has been developed for the propagation of *D. pentacheta*, a perennial species with potential for use in borders or as a groundcover in low-maintenance landscapes. *Dyssodia pentacheta* cuttings from stock plants or in vitro-grown shoots were easy to root under an intermittent mist system, but benefited from a 3- to 30-mM IBA basal dip. In vitro-grown explants produce more shoots per explant when cultured on WPM with 1 to 10 μM BA, rather than a combination of BA and 0.5 μM NAA. A reduced sucrose concentration in WPM was found to maintain plants in vitro, which suppressed phenolic production and allowed axillary shoots to grow from nodal explants. Tissue culture would be beneficial for rapid multiplication of new stock material and for release of new cultivars of *Dyssodia.* Potential may exist for applying these propagation techniques to *Dyssodia tephroleuca* Blake, an endangered species.

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