

# Micropropagation of the Rare Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*)

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**Abstract.** Shoot tip and stem segment explants collected from greenhouse-maintained plants of *Hymenoxys acaulis* var. *glabra* were cultured *in vitro* for shoot initiation on a Murashige and Skoog (MS) medium supplemented with 30 g·L<sup>-1</sup> sucrose, 2.5 μM BA, and 7 g·L<sup>-1</sup> agar at a pH of 5.7. Unbranched shoot explants were subcultured to MS medium with 0.0, 0.5, 1, 2, 4 or 8 μM BA for shoot proliferation. A maximum of 10.3 shoots per explant was produced on the medium with 2.0 μM BA. Nonrooted shoots were subcultured to MS medium with 0.0, 0.5, 2, or 8 μM K-IBA for rooting. Maximum rooting was 90% on MS medium with 0.5 μM K-IBA. Rooted shoots were greenhouse-acclimatized for 10 days. Overall survival was 75%. Chemical names used: 6-benzyl adenine (BA); potassium salt of indole-3-butyric acid (K-IBA).

*Hymenoxys acaulis* (Pursh) Parker var. *glabra* (Gray) Parker [also known as *Hymenoxys herbacea* (Greene) Cusick and *Tetranuris herbacea* Greene] is an herbaceous perennial known as the lakeside daisy (Fig. 1). Listed as threatened by the U.S. federal government and as endangered by the states of Illinois and Ohio (U.S. Fish and Wildlife Service, 1990), it is endemic to the Great Lakes region of North America, with populations in Michigan and Ohio in the United States, and in Ontario, Canada.

Natural stands of the lakeside daisy occurred historically in Illinois, but the last known population was destroyed in 1981. However, in the late 1970s three plants were collected from this population and maintained in a private garden until propagated for a restoration project (DeMauro, 1993). The plants were repeatedly cross-pollinated for 6 years, but failed to produce seed. This species is self-incompatible, so the Illinois plants are presumably of the same mating type (DeMauro, 1993). Therefore, the genetic integrity of the remaining Illinois plants can only be maintained through vegetative propagation.

The lakeside daisy forms a persistent basal rosette that spreads vegetatively from rhizomes. It can be propagated by division or rooted cuttings. However, its creeping rootstock limits the number of divisions or cuttings that can be taken from a single plant. Plants can be difficult to maintain in a garden setting or in pots, as they are sensitive to poor soil drainage and overwatering. Therefore, a micropropagation study utilizing the Illinois genotype was initiated as an alternative means of propagating the lakeside daisy. To my knowledge there are no other reports on the micropropagation of this taxon.

## Materials and Methods

Container-grown plants of the Illinois genotype were obtained from The Morton Arboretum (Lisle, Ill.) in Aug. 1998 and maintained in a greenhouse. Individual shoots up to 5 cm in length were removed in July and Aug. 1999, and all fully expanded leaves and most of the leaf primordia were removed. Stem segments were surface-disinfested in 1.0% sodium hypochlorite containing 0.1% Tween 80 for 12 min and then rinsed twice for 10 min total in sterile deionized water. Explants consisting of shoot tips or stem segments (each with three to five nodes) ≈ 1 cm in length were individually placed basal end down in 25 × 150-mm culture tubes containing 10 mL of MS basal salts and vitamins (Murashige and Skoog, 1962), 30 g·L<sup>-1</sup> sucrose and 2.5 μM benzyladenine (BA).

Medium pH was adjusted to 5.7 prior to adding 7.0 g·L<sup>-1</sup> Sigma A 1296 agar (Sigma Chemical Co., St. Louis). Culture tubes were capped with polypropylene caps and autoclaved at 121 °C for 15 min.

Capped culture tubes were sealed with Parafilm (Pechiney Plastic Packaging, Menasha, Wis.), then placed upright and maintained at 22 °C under a 14-h photoperiod with a photosynthetic photon flux (PPF) of 15 to 40 μmol·m<sup>-2</sup>·s<sup>-1</sup> [measured with a LI-190SA Quantum Sensor (LI-COR, Lincoln, Nebr.)] provided by two 40-W cool-white fluorescent lamps.

The primary explants were cultured for 6 weeks; then the original shoot segments and axillary shoots were excised and recultured every 4 weeks for six to eight culture periods prior to the rooting study and for 8 to 11 culture periods prior to the shoot proliferation study. Leaves were removed from the lower half of each explant with each subculture as they curled downwards and pushed the explants out of the medium. Individual, unbranched shoot explants (2 cm) were cultured on MS medium with 0, 0.5, 1, 2, 4, or 8 μM BA for shoot proliferation. After 4 weeks of culture, shoots were harvested, and the number of axillary shoots recorded. Shoots (2–3 cm) were rooted on MS medium with 0, 0.5, 2, or 8 μM of the potassium salt of indole-3-butyric acid (K-IBA). Percent rooting and the number of roots per shoot were recorded after 4 weeks.

Individual shoots were rooted on MS medium with 0.5 μM K-IBA for 20 d, rinsed free of medium, and planted in a 72-cell plug tray containing 1 Turface® (Profile Products LLC, Buffalo Grove, Ill.): 1 fine sand : 1 perlite : 2 Strong-Lite Universal Mix (Strong-lite Products Corp., Pine Bluff, Ark.) (by volume). The tray was covered with a clear plastic dome and placed in a shaded greenhouse on 1 Aug. 2000. The dome was removed after 3 d, and the tray moved to the edge of a shaded mist bench for 1 week, and then to an open greenhouse bench.



Fig. 1. The lakeside daisy (*Hymenoxys acaulis* var. *glabra*) growing *inter situ* at The Morton Arboretum, Lisle, Ill.

Percent survival was recorded 30 d after plants were removed from tissue culture. This study was conducted once, utilizing 80 explants.

Forty explants were randomly assigned to each shoot proliferation and rooting treatment. Experiments were conducted twice (n = 80). Data were subjected to analysis of variance, and means separated according to the Student–Newman–Keul test (Sokal and Rohlf, 1969). Percent rooting was analyzed with the G statistic, comparing the individual auxin treatments to the control (no K-IBA). Data were analyzed with CoStat statistical software (CoHort Software, Berkeley, Calif.).

## Results and Discussion

Explants on BA-free medium produced one unbranched shoot each, indicating strong apical dominance (Table 1). BA stimulated the production of axillary shoots regardless of concentration. BA at 2  $\mu\text{M}$  induced significantly more axillary shoots (10.3 shoots per explant) than did the other treatments (Table 1). BA has been utilized to stimulate axillary shoot production in other taxa of the Asteraceae. In comparison, optimal shoot proliferation rates were 5.3 shoots per single node explant as induced by 4.43  $\mu\text{M}$  BA in *Achillea asplenifolia* VENT. (Wawrosch, 1994); 19.8 shoots per shoot explant as induced by 2.7  $\mu\text{M}$  BA in *Liatris spicata* (L.) Willd. (Stimart and Harbage, 1989); and 7.2 shoots per single node explant as induced by 3  $\mu\text{M}$  BA in *Dyssodia pentacheta* (D.C.) Robins (Zimmerman et al., 1991). BA appears effective in stimulating axillary shoot proliferation in taxa of the Asteraceae, but it is recommended that a range of concentrations be tested to optimize shoot production.

Treatment of *Hymenoxys acaulis* var. *glabra* shoot explants with 4 or 8  $\mu\text{M}$  BA stimulated significantly more shoots than did the control, but also stimulated basal callusing on most of the explants (Table 1). Therefore, these and higher concentrations of BA are not recommended for shoot proliferation in this taxon, as callus production could interfere

Table 1. Shoot production from single shoot explants of *Hymenoxys acaulis* var. *glabra* after 4 weeks of culture on MS medium with various BA concentrations.

BA ( $\mu\text{M}$ )	Mean no. shoots/explant	Callus <sup>z</sup>
0.0	1.0 d <sup>y</sup>	–
0.5	5.2 c	–
1.0	7.4 b	–
2.0	10.3 a	–
4.0	8.9 b	+
8.0	8.6 b	+

<sup>z</sup>Callus present (+) or absent (–).

<sup>y</sup>Mean separation by Student–Newman–Keul test,  $P \leq 0.05$ ; n = 78 to 80.

with subsequent rooting and acclimatization.

K-IBA at all three concentrations tested was significantly more effective in inducing rooting than no K-IBA (Table 2). Maximum rooting was 90% for the shoots treated with 0.5  $\mu\text{M}$  K-IBA. The mean number of roots per shoot was significantly increased by treatment with 0.5 or 2.0  $\mu\text{M}$  K-IBA in comparison with the other two treatments (Table 2). IBA is effective for the rooting of other members of the Asteraceae. *Centaurea junoniana* shoots rooted 100% when treated with 0.05  $\mu\text{M}$  IBA (Hammatt and Evans, 1985), and the greatest number of roots produced per shoot for *Liatris spicata* was stimulated by 5  $\mu\text{M}$  K-IBA (Stimart and Harbage, 1989).

Of the 69 rooted shoots removed from tissue culture, 52 (75%) survived the greenhouse acclimatization process. The initial utilization of a plastic dome over the rooted shoots may have kept the relative humidity (RH) and/or temperature too high for this taxon, as plants began senescing within the first 3 d; once the plastic dome was removed and the plants placed in an environment with lower RH and temperature, plant losses diminished. Seven months after removal from tissue culture, the plants visually appeared very uniform.

Plants of the Illinois genotype are being utilized for display and restoration research at both The Morton Arboretum and at Chicago Botanic Garden. Propagation and maintenance

Table 2. Percent rooting and the number of roots produced per rooted shoot from shoot explants of *Hymenoxys acaulis* var. *glabra* after 4 weeks of culture on MS medium with K-IBA.

K-IBA ( $\mu\text{M}$ )	Rooting (%)	Mean no. roots/rooted shoot
0.0	68	3.1 b <sup>z</sup>
0.5	90**	17.3 a
2.0	86*	18.3 a
8.0	85*	5.0 b

<sup>z</sup>Mean separation by Student–Newman–Keul test,  $P \leq 0.05$ ; n = 68 to 75.

\*. \*\*Significantly different from the control at  $P \leq 0.05$  or 0.01, respectively, by the G statistic.

of this germplasm is a conservation priority. Micropropagation offers an alternative means of propagating the rare plant *Hymenoxys acaulis* var. *glabra*. I recommend 2.0  $\mu\text{M}$  BA for shoot multiplication, 0.5  $\mu\text{M}$  K-IBA for rooting, and moderate RH for subsequent acclimatization of rooted shoots.

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