Micropropagation of Antelope Bitterbrush [*Purshia tridentata* (Pursh) DC.]

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Abstract. In vitro-derived microshoots of antelope bitterbrush, incubated for 1 month in media supplemented with 0.44 μM BA, grew 0.8 and 1.1 cm longer in woody plant medium (WPM) compared to full-strength and half-strength Murashige and Skoog (MS) media, respectively. Explants cultured in WPM supplemented with 0.44 μM BA and 0.54 μM NAA produced a mean of five axillary shoots per explant. Explants dipped in 0.1% IBA or 0.1% NAA rooted best in 0.1% IBA with 89% success ex vitro vs. 60% success in vitro. Survival of acclimatized plantlets rooted ex vitro was 95%, while 50% survived when rooted in vitro. After 1 year of greenhouse growth, 98% of plantlets survived and flowered. Chemical names used: benzyladenine (BA), 3-indolebutyric acid (IBA), 1-naphthalene-acetic acid (NAA).

Antelope bitterbrush is a genetically diverse rosaceous shrub of high nutritive value to browsing animals of rangelands and forests in the western United States (Nord, 1965; Welch et al., 1983; Winward and Findley, 1983), but overgrazing, fire, and introduced grasses have reduced its distribution (Ferguson and Medin, 1983). Low seed yield often slows natural recovery; seed dormancy and rodents frequently hamper seedling establishment following artificial regeneration by direct seeding (Young and Evans, 1981). Vegetatively propagated planting stock could help regenerate nonreproductive populations, and because some genotypes resprout after fire (Martin and Driver, 1983), depleted habitat could be revegetated with fire-resistant clones. Antelope bitterbrush, with its profuse aromatic yellow spring bloom, also has potential ornamental use in xeric landscapes.

Although some antelope bitterbrush layer naturally (Nord, 1965), stem cuttings do not root readily (Everett et al., 1977). Kyle and Righetti (1988) micrografted shoot-tip scions [grown in a modified Murashige and Skoog (MS) (1962) medium supplemented with 8.8 μM BA and 0.49 μM IBA] onto seedling root-

stock in vitro. They did not, however, optimize proliferation or root shoot tips.

Shoot multiplication by repeated induction of axillary buds can regenerate true-to-type plantlets (George, 1993). Minimal culture time and reduced use of growth regulators limit undesirable adventitious shoot growth and variation in culture. This study examined in vitro axillary shoot culture of antelope bitterbrush because a protocol to mass propagate it had not as yet been developed. Our experiments aimed to optimize the basal medium, identify concentrations of growth regulators adequate to produce true-to-type plantlets, assess in and ex vitro rooting, and observe plant survival and growth in the greenhouse.

Materials and Methods

Plant material and culture conditions. Softwood cuttings (8 cm long) of 46 wild genotypes of antelope bitterbrush were collected directly from shrubs, or produced from seed germinated from plants that resprouted following wildfire (Table 1). The seedlings were grown for 2 years in pots under natural light at 20 to 25 °C in a greenhouse in Moscow, Idaho.

Basal leaves were removed from freshly harvested shoots, leaving only small leaves near the shoot-tip. Defoliated shoot-tips were agitated continuously for 10 min in 1000 mL of an aerated aqueous solution containing five drops of detergent (Liquinox, New York), surface-disinfested for 15 min in a continu-

ously stirred 1% (w/v) NaOCl solution, and rinsed three times in sterilized deionized water. MS medium was supplemented with 0.44 μM BA and 0.42% Agargel (Sigma Chemical Co., St. Louis), dispensed into 25 × 150-mm glass test tubes (10 mL/tube), and autoclaved for 20 min at 120 °C and 100 kPa. Bases of shoot-tips were recut transversely and 1.5-cm-long explants were inserted upright into the medium and covered by loose-fitting cotton-filled caps (Magenta Co., Chicago). Cultures were grown in a room at 22 °C night/27 °C day under an 18-h photoperiod (20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at culture level outside the test tube) provided by 40-W cool-white fluorescent lights. Experiments used shoots derived from up to 20 monthly subcultures of all genotypes. Multiplication data were collected after 1 month of culture.

Optimizing the basal medium. The influence of the following basal salts on shoot proliferation and growth was compared: MS, 1/2MS, and woody plant medium (WPM) (Lloyd and McCown, 1980). Microshoot explants (1.5 cm long) were assigned to three replications (30 explants per replication) of these media with and without 0.44 μM BA in a randomized complete-block (RCB) design. Data were recorded on shoot elongation and proliferation rate.

Shoot proliferation. A 2 × 2 factorial experiment was conducted to determine effects of BA and NAA levels on microshoot elongation and multiplication. Explants (0.5 cm long) were grown (45 explants per treatment) in WPM with 0.44 μM BA and 0.54 μM NAA in a completely randomized (CR) design. Length and counts of axillary and adventitious shoots were recorded.

In vitro rooting. Because continuous auxin exposure in prior trials inhibited rooting, explants were subjected to 5 min water-based dips, 88 explants per treatment, in 0% or 0.1% IBA or in 0.1% NAA in a CR design. Explants (1.5 to 2 cm long) were cultured in WPM without growth regulators and placed in a growth chamber at 18 °C night/20 °C day, with lighting as described previously. Rooting was assessed for 8 weeks.

Ex vitro rooting. Microshoots (1.5 to 2 cm long) were cultured for 1 month in WPM without growth regulators, removed from culture tubes, soaked for 20 s in benomyl (methyl 1-[butylcarbomoyl]-2-benzimidazolecarbamate) fungicide at 1 g·L⁻¹, and dipped in talc powder with 0.1% IBA or 0.1% NAA. Three replications (40 shoots per replication) were used in a RCB design. Shoots were set in 5 mL capacity plastic cells containing a sterile medium of 2 perlite : 1 peat : 1 vermiculite (by volume) and placed in 88% minimum relative humidity provided by fog, 60% shade, 15 °C

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Table 1. Sources of antelope bitterbrush used in propagation experiments.

Material collected	Geographical location ²	Populations sampled (n)	Genotypes per population (n)
Cuttings	Ochoco NF, Ore.	7	4
Cuttings	Franklin Co., Wash.	3	4
Seed	Cassia Co., Idaho	3	1
Seed	Humboldt NF, Nev.	3	1

²NF = National Forest.

Table 2. Effects of BA and NAA levels on multiplication of in vitro-derived microshoots of antelope bitterbrush.^a

Growth regulator (μM)		Shoots/explant		Shoot ht ^a
BA	NAA	Axillary ^b	Adventitious	(cm)
0	0	0.4 a	0.1 a	0.3 a
0	0.54	0.2 a	0.1 a	0.4 a
0.44	0.00	3.9 b	0.2 a	1.4 b
0.44	0.54	5.0 c	0.7 b	2.1 c

^aForty-five microshoots were used per treatment. Mean separation in columns by Tukey's HSD at $\alpha = 0.05$.

^bBA effect ($P = 0.0001$), NAA effect ($P = 0.0457$), NAA $\times$ BA interaction ($P = 0.0031$).

^cBA effect ($P = 0.0001$), NAA effect ($P = 0.0001$), NAA $\times$ BA interaction ($P = 0.0183$).

night/25 °C day air cycles, a root zone at 20 to 22 °C, and natural photoperiod. Rooting was assessed after 4 and 8 weeks.

Plantlet survival and growth. A total of 334 in vitro- and 310 ex vitro-rooted microshoots were transferred to a greenhouse in February. Survival was tallied 1 month later. In March, 50 plantlets were transplanted to 215-mL capacity polystyrene containers filled with 1 perlite : 1 vermiculite : 1 peat (by volume). Plantlets were grown under natural light at 15 to 25 °C and fertilized twice weekly for 1 month with 7N-40P-17K with N at 50 mg·L⁻¹, and then for 2 months with 20N-20P-20K with N at 200 mg·L⁻¹. Stem length, number of stems, and flowering habit were recorded on 30 plants after 1 year.

Data analysis. Confidence intervals expressed uncertainty of means at $P \leq 95\%$. If Pearson correlation indicated dependent variables, multivariate analysis of variance MANOVA (SAS Institute, 1989) was used, and analyses of variance (ANOVA) were conducted when Wilks-lambda statistics had significance <0.05 . Non-normal distributions un-

derwent the Kruskal-Wallis test or Friedman two-way ANOVA. Categorical data were examined by maximum likelihood ANOVA using CATMOD (SAS Institute, 1989) and single degree-of-freedom contrasts.

Results

Optimizing the basal medium. Explants cultured in growth regulator-free MS, 1/2MS, and WPM developed dark-green rosettes, whereas explants supplemented with 0.44 μM BA produced extended light-green foliage. Because explants grew up to 1.1 cm taller on WPM vs. 1/2MS ($P = 0.0008$) and developed five more shoots per explant on WPM vs. MS ($P = 0.0002$) (Fig. 1), WPM was selected for later experiments. A slow-growing bacterium contaminated some explants, but was not present in later cultures.

Shoot proliferation. The best treatment, 0.44 μM BA plus 0.54 μM NAA, resulted in 0.7 more axillary shoots per explant and 1.1 cm taller shoots than BA alone (Table 2). Although NAA alone had little effect on number

of shoots produced, its interaction with BA increased axillary shoot proliferation. A few adventitious shoots developed on basal callus.

In vitro rooting. Fleishy roots emerged from basal callus after 2 to 5 weeks culture. The 5-min soak in auxin improved rooting success of in vitro shoots ($P < 0.01$). After 5 weeks, controls and shoots treated with 0.1% NAA or 0.1% IBA had rooted with 27%, 40%, and 60% success, respectively. Media discolored and roots blackened in cultures that were not transplanted ex vitro soon after root emergence.

Ex vitro rooting. Shoots rooted between 2 and 8 weeks after transfer ex vitro. Controls and shoots treated with 0.1% NAA or 0.1% IBA rooted best with 52%, 65%, and 72% success, respectively, after 4 weeks ($P = 0.0368$) and with 92%, 89%, and 79% success, respectively, after 8 weeks. Plantlets developed an average of 3.4 ± 0.5 roots per microshoot.

Plantlet survival and growth. A total of 167 (50%) in vitro-rooted and 294 (95%) ex vitro-rooted plantlets survived acclimatization. After one season of greenhouse growth, 98% of transplanted plantlets survived and produced leaders which extended 16.4 ± 1.6 cm with 1.3 ± 0.2 branches per plantlet. Plantlets developed upright and prostrate forms and produced an average of 20 flower buds per leader the following spring.

Discussion

Successful micropropagation of antelope bitterbrush genotypes from various geographical sites suggests that this diverse species can be increased by vegetative means. Plantings, however, should be locally adapted and contain many individuals within population ecotypes (Meyer and Monsen, 1992), even if some genotypes grow sub-optimally in vitro.

Optimal culture of antelope bitterbrush in low salt medium (WPM), supplemented with 0.44 μM BA plus 0.54 μM NAA, produces at least three more shoots per explant than the minimal level (two) adequate for mass propagation (de Fossard, 1994). Small (0.5 cm) explants used in the shoot proliferation experiment, however, likely limited shoot growth compared to that produced in the basal medium test. A taxon closely related to bitterbrush, *Cowania subintegra* Kearney (Arizona cliffrose), was cultured in high salt MS medium with BA plus IBA, but unlike antelope bitterbrush, *C. subintegra* is insensitive to BA alone (Jakobek et al., 1993).

Improved rooting success and plantlet survival of ex vitro- vs. in vitro-rooted bitterbrush is consistent with impaired function of in vitro-formed roots in other species (Smith and McClelland, 1991). IBA or NAA accelerate ex vitro rooting, but untreated microshoots root equally well after 8 weeks.

Flowering of plantlets (donor age 2 years plus 1 year plantlet growth) is consistent with first bloom of seedlings after 3 years growth under favorable conditions (Nord, 1965). Thus, plantlets could potentially produce seed sooner on harsh sites where seedlings may take up to 10 years to flower.

Fig. 1. Growth of antelope bitterbrush microshoots on WPM, MS, and 1/2MS media; all supplemented with 0.44 μM BA. Vertical bars represent 95% confidence intervals of means (sample size = 150).

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