Protocols for Mass Micropropagation of Antelope and Desert Bitterbrush

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Abstract—Vegetative propagation of Purshia tridentata and P. glandulosa could potentially capture and multiply valuable genetic traits such as fire resistance and produce plants which reproduce in a shorter time than seedlings. The goal of this research was to develop mass propagation protocols for antelope and desert bitterbrush using micropropagation techniques. Microshoot tips were incubated in vitro on alternative nutrient media supplemented with different levels of cytokinin and auxin growth regulators. An average of 7.9 shoots/explant of P. tridentata were produced after 1 month on Woody Plant Medium (WPM) supplemented with 0.1 mg/l benzlyadenine (BA). P. glandulosa multiplied 5-fold over 1 month on the same media. Best rooting occurred ex vitro for both species. P. tridentate microshoots rooted at 88.6% after treatment with Hormex® #1 (0.1% IBA) and P. glandulosa microshoots rooted up to 78% after treatment with Hormex® #3 (0.3% IBA). Flowering and fruiting of micropropagated plantlets from both species occurred after one season's growth in the greenhouse. These successful mass propagation systems for bitterbrush could play an important role in range restoration.

INTRODUCTION

Antelope and desert bitterbrush *(Purshia tridentata* (Pursh) DC. and *P. glandulosa* Curran, respectively) are roseaceous, nutritive forage shrubs in the semi-arid rangelands of Utah, Nevada, Idaho and California (Welch et al., 1982). Wild-fires and livestock overgrazing have degraded range habitat and reduced the population of both bitterbrush species. Since efforts to propagate material for revegetation projects have been hindered by seed dormancy and seed predation (Young and Evans, 1981), vegetatively propagated plants could provide an alternative and more reliable source of planting stock. Micropropagated antelope and desert bitterbrush plants, produced in a relatively short time, could potentially restore damaged habitat with fire-resistant, palatable, nutritious and drought-hardy selections.

This paper describes mass-propagation protocols for antelope and desert bitterbrush and evaluates the effects of culture media and plant growth regulators on multiplication and rooting.

GENERAL METHODS

Culture initiation

Our source of *P. tridentata* and *P. glandulosa* material was wild seed, collected from rangeland shrubs in southern Idaho

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and Nevada, respectively. Seedlings were grown in pots at the University of Idaho Plant Science greenhouse. Shoot tips of new growth were collected from 2-yr-old containerized seedlings and placed in sterile culture for propagation.

The cuttings were washed in running tap water for 30 min. Leaves were excised from the stems, and the defoliated shoots were agitated for 10 min in an aerated aqueous solution containing 5 drops of Tween® 80 per liter. The stems were then surface sterilized for 15 min in a continuously stirred 20% Clorox® bleach solution (1% NaClO) and rinsed 3X in sterile, distilled, deionized water. The stem segments were given fresh basal cuts and placed individually in 25 X 150 mm test tubes containing 10 ml of Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962). These and all subsequent cultures grew on open shelves under an 18-h photoperiod provided by cool-white fluorescent lights (40 W) which produced approximately 20 umoles m^{-2} s^{-1} PAR on the leaf surfaces. Diurnal temperatures ranged from 22° to 27°C.

To increase the number of microshoots available for experiments, stems were cut into 2-node segments and transferred to fresh media monthly until sufficient numbers of uniform healthy microshoot tips were produced.

Selecting a culture medium

Although MS medium has commonly been used as a substrate for micropropagation, many woody plants grow better on other defined media with lower concentrations of mineral nutrients (Bonga and von Aderkas, 1992) such as Woody Plant Medium (WPM) (Lloyd and McCown, 1980). Hence, we cultured microshoots on MS and WPM media to detect differences in shoot elongation and the number of axillary shoots produced.

Shoot multiplication

Microplants exposed to potent and high levels of cytokinins in culture media often produce adventitious as well as axillary shoots (Huetteman and Preece, 1993), and adventitious shoot production has been linked to increased rates of somaclonal variation (Pierik, 1987). To decrease the possibility of adventitious shoot formation, thus, maintaining the genotypes of the bitterbrush shoots, the cytokinin benzyladenine (BA) was used at low to moderate levels for shoot multiplication.

Ex vitro rooting

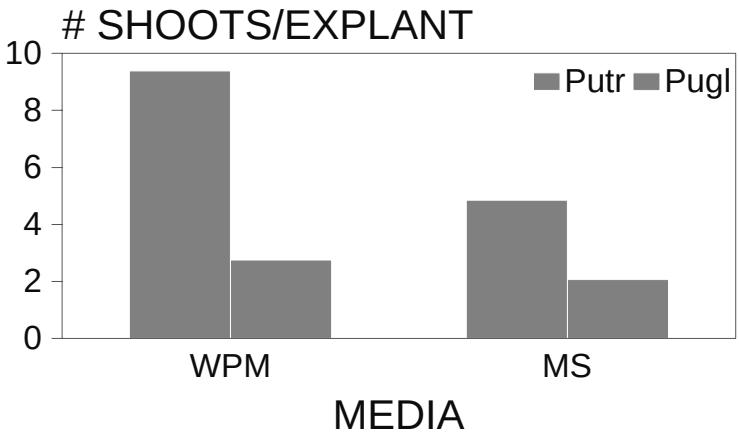
The effect of several commercial auxin preparations on the rooting of microshoots was assessed after 6 weeks growth under fog humidification on a greenhouse propagation bench. The auxin treatments were either basal talc dips in: Hormex® #1 (0.1% indolebutyric acid (IBA)); Hormex® #3 (0.3% IBA); Hormex® #8 (0.8% IBA); or Rootone® (0.2% naphthaleneacetic acid (NAA) plus 4.04% Thiram®); or a 5-sec dip in a 1:10 solution of Dip 'n Grow® (0.1% IBA and 0.05% NAA). All microshoots were immersed in 1 g/l of the fungicide benomyl for 20 sec before being treated and planted. The treated microshoots were placed on propagation benches under 60% shade and natural photoperiod. Root zone temperatures varied from 20° to 22°C, relative humidity from 86 to 92%, and diurnal air temperatures from 15° to 25°C.

PROPAGATION OF *PURSHIA TRIDENTATA*

Materials and methods

Culture medium: 90 microshoots were equally assigned to hormone-free MS, WPM and ½ MS. To compare microshoot elongation and new shoot production on the same 3 media containing BA, 450 shoot tips were randomly assigned to WPM, MS or ½ MS, each supplemented with 0.1 mg/l BA. After 4 weeks, microshoot elongation, the number of new shoots and health scores were recorded for each explant.

Shoot multiplication: 270, 1.5-cm-long microshoot tips were assigned in equal numbers to 9 concentrations of BA (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8,



1.6, and 3.2 mg/l) on WPM, arranged in a CR design. After 4 weeks, shoot elongation and counts of all new shoots were recorded.

In vitro rooting: 200 microshoots were assigned to 2 replicates of 4 auxin treatments arranged in a RCB design. The treatments included a 5-sec NAA (1,000 mg/l) basal dip and culture on hormone-free WPM, and placement on WPM containing 0, 0.1 and 1.0 mg/l NAA. Rooted microshoots were tallied after 4, 6 and 8 weeks. In a second experiment, 432 1-cm-long microshoots were either dipped or not dipped in 1,000 mg/l NAA and placed on ½ MS or WPM.

Ex vitro rooting: 480, 1-cm-long microshoots were removed from test tubes and assigned to 3 replicates of a control (no hormone), Hormex® #1, Rootone® and Dip 'n Grow® arranged in a RCB design. The treated shoots were struck into 5-ml capacity miniplug cells containing a 2:1:1 mix of perlite, peat and vermiculite (v/v).

Acclimatization: 50-in vitro-rooted microshoots with normal root development were transplanted to 215 cc capacity cells filled with a 1:1:1 mix of perlite, vermiculite, and peat (v/v). The plants grew under natural light with greenhouse temperatures varying from 15° to 25°C during the growing season. Survival, shoot growth, number of stems, and flowering habit were recorded 1 year later on a random sample of 30 survivors.

Results and discussion

Culture Medium: After 1 month in culture, the microshoots on BA-free MS, ½ MS and WPM elongated an average of only 4 mm and produced no new shoots. In contrast, microshoots on the same 3 media supplemented with 0.1 mg/l BA lengthened rapidly and produced vigorous new shoots. Greatest stem elongation and new shoot production occurred on WPM. The microshoots grew an average of 0.8 and 1.1 cm taller on WPM versus MS and ½ MS, respectively ($p = 0.0008$), and developed an average of 5 more new shoots per explant on WPM versus MS ($p = 0.0002$).

The new shoots on WPM developed normal healthy foliage (mean health score 2.3) versus slightly more chlorotic growth on MS (2.1), and ½ MS (1.4) media ($p = 0.005$). Because the bitterbrush microshoots grew more vigorously on WPM than on either MS or ½ MS, we selected WPM as the medium for subsequent in vitro experiments.

Shoot Multiplication: New shoots developed from axillary nodes and the bases of explants treated with varying levels of BA. In contrast, the explants on BA-free medium were stunted and failed to release axillary shoots. The number of new shoots increased from 0 on hormone-free medium, to a maximum average of 15, after treatment with 0.8 mg/l BA, and then declined at higher concentrations of BA. Maximum stem growth (1.8 cm) occurred with treatment of 0.1 mg/l BA, and decreased significantly after treatments > 0.2 mg/l BA ($p = 0.0001$). The difficulty of clearly identifying axillary shoots increased when BA concentrations rose above 0.2 mg/l, due to shorter stem internodes and increased basal shoot proliferation. Therefore, the maximum shoot count of 15 may include adventitious shoots less desirable for clonal production. The BA level of 0.1 mg/l resulted in optimal elongation, a moderate number of new shoots

produced, and reasonable assurance of the axillary nature of the new shoots.

In vitro rooting: NAA seemed to inhibit normal rooting. Microshoots on NAA-free WPM (control) developed white "normal" roots which penetrated to the bottom of the test tube. Explants on the NAA-supplemented media, however, developed "aerial" horizontal fibrous roots on the surface of the medium and produced few if any normal roots. In the second experiment, 11% of the NAA-treated microshoots rooted versus 53% of the non-treated explants ($p < 0.0001$) after 6 weeks in culture. Rooting was similar on both WPM and ½ MS incubation media.

Ex vitro rooting: Microshoots rooted more successfully ex vitro than in vitro (Fig. 1). After 6 weeks, 62% of the untreated microshoots (control) had rooted versus 88.6% and 79.0% of the microshoots treated with Rootone® and Hormex® #1, respectively. The microshoots treated with Dip 'n Grow® rooted at 65.3%. We infer that a low rate of auxin, applied as either IBA or NAA powder treatment, will likely result in ex vitro rooting at a level sufficient for commercial production.

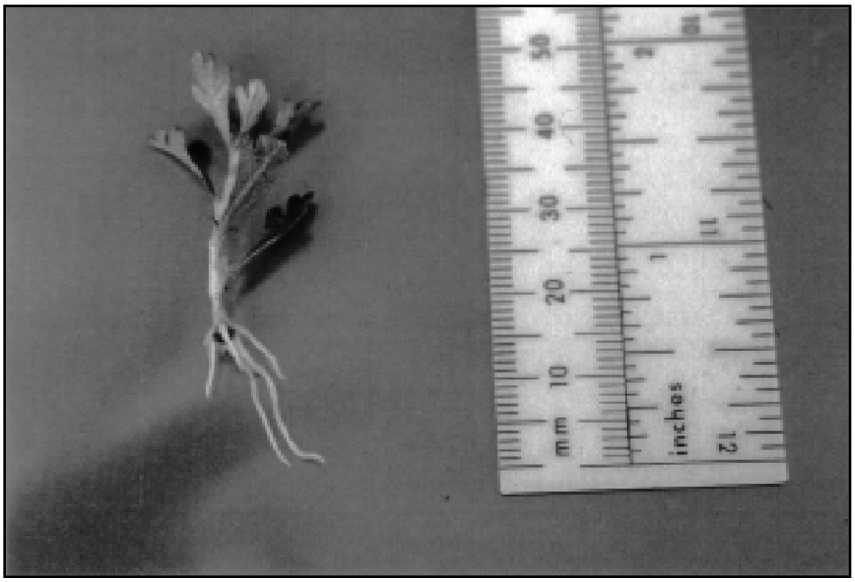


Figure 1. Ex vitro rooted *Purshia tridentata* plantlet 4 weeks after treatment with 0.1% IBA.

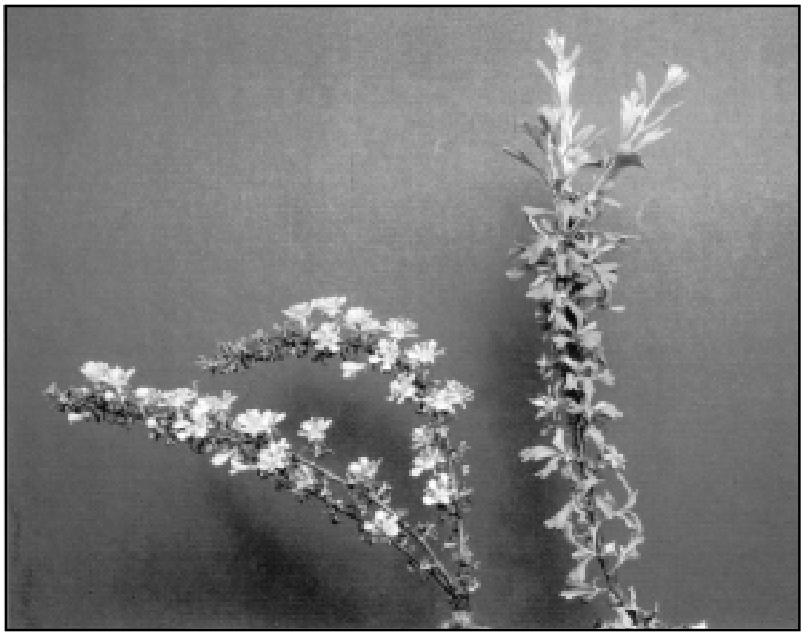


Figure 2. Flowering desert bitterbrush (left) and antelope bitterbrush (right) after 1 year in the greenhouse.

Acclimatization: Four months after transplant, 85% of the plantlets remained alive with no subsequent death. By the end of the first year's growth in the greenhouse, the plantlets had produced leaders with an average extension of 16.4 cm. The plantlets bloomed heavily within 1 month of budbreak with an average of 20 flower buds on each leader (Fig. 2). The precocious and prolific flowering of the plantlets suggests that regeneration could occur the first year after outplanting this type of planting stock if conditions are favorable for establishment.

PROPAGATION OF *PURSHIA GLANDULOSA*

Materials and methods

Culture medium: 360, 2-cm-long shoot tips were excised and placed randomly on MS or WPM containing 0.1 mg/l BA. After 5 weeks, we calculated stem elongation and counted the number of axillary shoots produced.

Shoot multiplication: 160 shoot tips were randomly assigned to 24 treatment combinations of MS + 0, 0.05, 0.1, 0.2, 0.4, 0.9, 1.8 or 3.6 mg/l BA. Twenty microshoots were used per treatment. After 4 weeks, shoot elongation and the number of axillary bud breaks were recorded for each shoot.

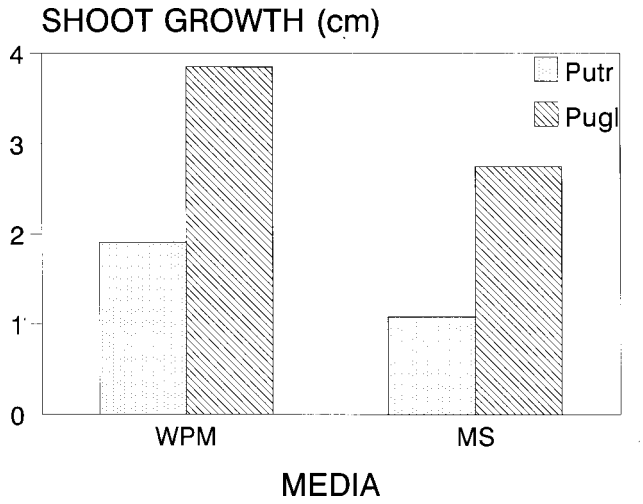


Figure 3. Effect of Woody Plant Medium (WPM) and Murashige and Skoog medium (MS) on shoot elongation of *P. tridentata* (Putr) and *P. glandulosa* (Pugl) microshoots after 4 weeks in culture.

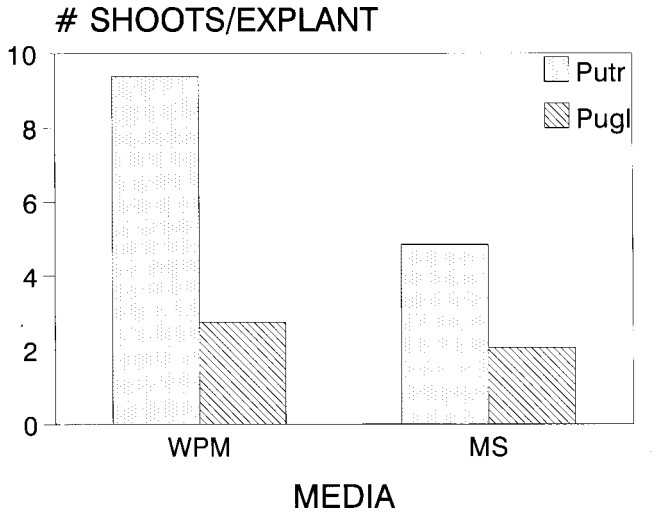


Figure 4. Effect of Woody Plant Medium (WPM) and Murashige and Skoog medium (MS) on shoot production of *P. tridentata* (Putr) and *P. glandulosa* (Pugl) microshoots after 4 weeks in culture.

In vitro rooting: 216, 2-cm-long microshoots were divided among 6 media (MS, ½ MS and WPM supplemented with or without 1 mg/l NAA). Rooting was recorded after 4, 6, 8 and 10 weeks.

Ex vitro rooting: Non-lignified microshoots were cut into 600, 3-cm-long shoot tips and 600 stem segments. Forty tips and 40 segments were randomly assigned to each of 5 hormone treatments: a control (no hormone), Hormex® #3,

Hormex® #8, Rootone®, and Dip 'n Grow®. The experiment was replicated twice in a randomized split-plot design. The explants were struck in 5-ml capacity miniplug cells containing a 1:1 mix of Sunshine® #3 and perlite.

Acclimatization: 40 rooted microshoots were transferred from agar medium to 164 cc capacity Ray Leach® containers containing Sunshine® #2. Observations were made a year later on shoot growth, number and location of branches, and incidence of flowering and fruiting.

Results and discussion

Culture medium: Stem elongation and axillary shoot production were enhanced significantly on the WPM ($p<0.001$) versus MS medium (3.85 vs. 2.75 cm and 2.75 vs. 2.06 shoots, respectively). *P. glandulosa* microshoots developed taller stems but fewer axillary shoots than *P. tridentata* explants on both media (Figs. 3 & 4). Using a low level of BA (0.1 mg/l) would avoid the foliar chlorosis and adventitious shoot production associated with the higher levels of BA in the medium, a distinct advantage when propagating selected genetic traits.

Shoot multiplication: Microshoots developed tall, single stems on hormone-free medium (Fig. 5), in contrast to the stunted growth of *P. tridentata* on BA-free media. Explants achieved maximum growth with 0.1 mg/l BA (3.5 cm) and were shorter with more axillary bud break after treatments >0.2 mg/l BA. The number of new shoots increased from 0 on BA-free medium, to a maximum average of 5.0 shoots per explant after treatment with 0.4 mg/l BA, and then declined at higher concentrations of BA.

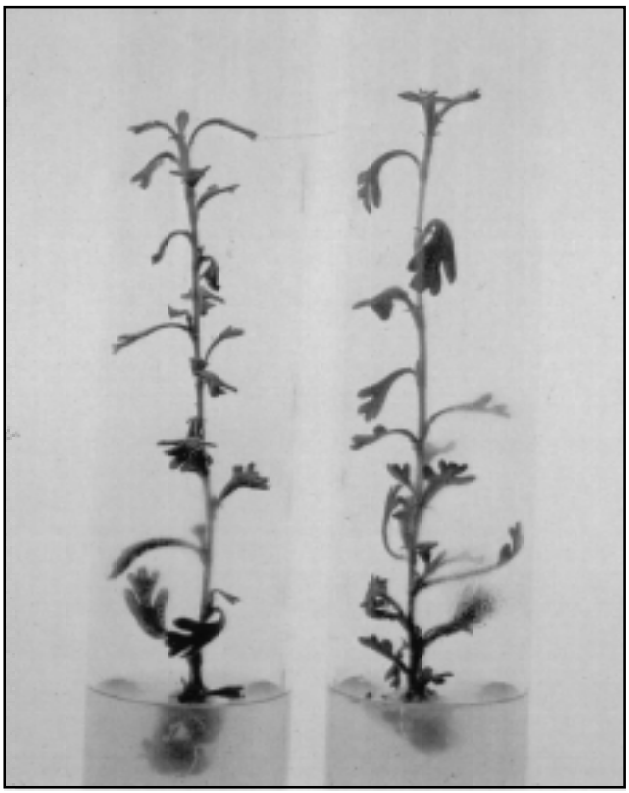


Figure 5. *Purshia glandulosa* microshoots after 1 month on hormone-free WPM. Note tall, single stem growth.

In vitro rooting: The best rooting (44%) occurred after 10 weeks on WPM + 1 mg/l NAA as compared to 0% and 19% rooting on NAA-supplemented MS and ½ MS, respectively. None of the microshoots on MS produced roots, regardless of whether NAA was present or not in the medium. This data suggests the most important factor in rooting is the basal medium. In contrast to the response of *P. tridentata*, NAA did not inhibit rooting.

Ex vitro rooting: Microshoot tips rooted most successfully after they were treated with a low concentration of auxin. After 6 weeks, 28% of the untreated microshoots had rooted versus 74%, 72%, and

71% of the microshoots treated with Hormex® #3, Hormex® #1 and Dip ‘n Grow®, respectively. Microshoots treated with Hormex® #8 rooted at 52%. Explant tips also rooted better than stem segments (60% versus 36%, respectively).

Acclimatization: Four months after transplant, 85% of the plantlets remained alive with no subsequent death. After 1 year in the greenhouse, the leading shoots had extended an average of 18.0 cm. Eighty-five percent of the plantlets flowered, with the axillary bloom generally extending to the full length of all the stems (Fig. 2).

CONCLUSIONS: THE PROPAGATION PROTOCOL

1. Initiating and multiplying both bitterbrush species on Woody Plant Medium, supplemented with 0.1 mg/l BA, provides numerous elongated healthy shoots suitable for multiplication in subculture.

2. Rooting the microshoots in ex vitro conditions with low levels of auxin would lower propagation costs, although untreated microshoots of *P. glandulosa* and *P. tridentata* rooted with moderate success under in vitro conditions (44% and 61%, respectively)

3. Plantlets of both species, transferred to greenhouse conditions, can provide flowering and fruiting plants within 1 year.

We have demonstrated that antelope and desert bitterbrush can successfully be propagated by micropropagation techniques, achieving numbers suitable for commercial production.

With micropropagated antelope and desert bitterbrush, range managers and scientists can potentially:

1. revegetate areas with locally selected genetic stock

2. shorten the time for breeding programs with selected clones

3. outplant material which could rapidly produce seed

4. lower the fire frequency, enhance forage, and increase cover of damaged habitat.

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