In Vitro Propagation of *Aconitum noveboracense* and *Aconitum napellus*

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Abstract. Shoot proliferation in vitro of a threatened species, northern monkshood (*Aconitum noveboracense* Gray), and of common European monkshood (*A. napellus* L.) was achieved using growth chamber-grown stem nodes cultured on Gelrite-solidified MS medium supplemented with 4.5 μM BA, whereas rooting occurred when BA levels were <3.4 μM. Problems with phenol oxidation and its diffusion into the medium were circumvented with addition of 0.1 μM citric acid and 500 mg liter⁻¹ PVPP. The use of 60 μM rifampicin provided effective control of most bacterial contaminants. Nonchimeric albino foliage was observed on 30% of shoots produced on agar. Seventy four percent of basal rosettes rooted directly in peat plugs under high humidity within 10 weeks. Normal growth morphology was exhibited after plants were subjected to a 10-week dormancy treatment (3°C, dark). Chemical names used: N-(phenylmethyl)-1H-purin-6-amine (BA); polyvinylpolypyrrolidone (PVPP).

*Aconitum noveboracense* has been federally designated as a threatened species. It is a perennial herb in the Ranunculaceae found in disjunct populations throughout the Paleozoic Plateau area of Iowa and Wisconsin as well as at several sites in Ohio and New York (5). The genus *Aconitum* is well-known as a source of pharmaceutically useful diterpene alkaloids (4).

*A. noveboracense* reproduces vegetatively through bulbul proliferation and the development of adventitious buds on lateral roots (1). A protocol for seed stratification that provides up to 95% germination only recently has been developed (2). Tissue culture is an alternative means of propagation that also provides for long-term germplasm preservation. Reported herein are procedures for in vitro propagation of *A. noveboracense* (threatened northern monkshood) and for a related species, *A. napellus* (common European monkshood).

*A. napellus* was growth chamber-grown from locally purchased first-year tubers. Stem nodes were washed and surface-sterilized by successive immersion in 70% ethanol for 1 min, 0.5% sodium hypochlorite plus 2 drops Tween 20 per 100 ml for 20 min followed by two rinses in sterile distilled water. Nodal segment ends were recut aseptically and the basal end of the nodes were placed in gellan gum or agar solidified medium.

The medium contained MS inorganic salts (3) plus the following: 87.6 mM sucrose, 0.1 μM citric acid, 0.9 mM L-threonine, 3.0 μM nicotinic acid, 2.4 μM pyridoxine-HCl, 13.3 μM glycine, 500 mg liter⁻¹ PVPP, 0–6.5 μM BA, and either gellan gum (Gelrite, Kelco, San Diego, Calif.) or agar (Bacto-agar, Difco). The medium was adjusted to pH 5.7 before autoclaving and was dispensed 7 ml per 30-ml vial (2.5 cm diameter × 5.5 cm high) or 30 ml per baby food jar (5 cm diameter × 10 cm high) using plastic closures or Parafilm. *Aconitum noveboracense* produced more phenolic oxidation and diffusion into the medium than did *A. napellus*. but oxidation could be controlled with citric acid and PVPP (6). The cultures were incubated at 25°C under low-intensity illumination (40 μmol s⁻¹ m⁻² from cool-white fluorescent lamps) with a 16-hr photoperiod.

The limited amount of growth chamber-grown *A. noveboracense* material necessitated the use of field-collected stems. Explants of field origin exhibited more than 90% contamination after surface sterilization. Inclusion of 60 μM rifampicin in the medium provided effective control of most bacterial contaminants in stage 1 cultures, making excision of sterile axillary shoots possible from field-collected stems (7). Nonchimeric albino foliage and reduced growth rates were noted on 30% of shoots on an agar medium. A change to the use of Gelrite gellan gum as the media solidifying agent alleviated the problem.

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Fig. 1. In vitro proliferation of basal rosettes from *Aconitum* explants. (a) Explants after 4 weeks of culture with 4.5 μM BA. (b) Individual rosettes excised from *A. noveboracense* explant shown on the left in a. (c) Four explants per container as cultured in vitro.
Table 1. Rosette production of \textit{Aconitum} as a function of subculture.\textsuperscript{2}

<table>
<thead>
<tr>
<th>Genotype</th>
<th>2</th>
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<th>8</th>
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<tbody>
<tr>
<td>\textit{A. noveboracense}</td>
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<tr>
<td>WCM\textsuperscript{3}</td>
<td>2.5 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>NA</td>
</tr>
<tr>
<td>HV</td>
<td>2.5</td>
<td>3.0 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>NA</td>
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<tr>
<td>WC</td>
<td>2.5</td>
<td>1.5 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0</td>
<td>2.7 ± 0.2</td>
<td>4.0 ± 0.6</td>
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<tr>
<td>\textit{A. napellus}</td>
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<tr>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>3.4 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>3.6 ± 0.4</td>
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\textsuperscript{2}Stage culture, 4.5 \textmu M BA, 4-week cycles.
\textsuperscript{3}± SE; NA = data not available.
\textsuperscript{4}Genotypes from Vernon County, Wis.: WCM = Wildcat Mountain, HV = Hay Valley, and WC = Weister Creek.

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Shoot proliferation occurred primarily in the form of a basal rosette (Fig. 1). Observations of 250 cultures grown at BA concentrations between 1 and 6.5 \textmu M indicated that cytokinin requirements for \textit{A. napellus} and \textit{A. noveboracense} were similar. The greatest number of usable rosettes for both species occurred on a medium containing 4.5 \textmu M BA (Fig. 2). At this BA concentration, an average of three usable rosettes developed from each explant during each subculture period of 4 weeks. At BA concentrations <3.4 \textmu M, most explants rooted spontaneously and explant proliferation rates were reduced. Explants placed on BA concentrations of 6.7 \textmu M or above displayed aberrant leaf morphology and reduced growth. Explants were subcultured to fresh medium every 4 weeks and have been carried through 10 subcultures (Table 1). There do not appear to be significant differences in rosette production between genotypes or species, and the rate of rosette production does not appear to decrease with time spent in culture.

Plantlets could be rooted in vitro by transferring rosettes to a medium without BA. Rooting occurred within 4 weeks, and plantlets were transplanted into a 3 sand : 1 potting soil mix (v/v) and placed in a high-humidity chamber for 2 weeks to acclimate. Plantlets also could be rooted by transferring rosettes directly to peat plugs. Survival depended on genotype and plantlet size. For the most successful genotype, 74\% of the rosettes rooted in a high-humidity chamber after 10 weeks. Both \textit{A. napellus} and \textit{A. noveboracense} produce thick, tuberous roots in vitro. Roots appear to be functional whether plantlets are rooted in vitro or directly in peat plugs, but the rooting response varies with genotype.

Plantlets were grown in an environmental chamber for 2 to 3 months. It was found that growth and flowering was stimulated by a 10-week dormancy treatment (3°C, dark). Subsequent growth morphology appeared normal.

This system can provide long-term germplasm storage and large numbers of cloned plants of two species of monkshood—\textit{Aconitum napellus} and the threatened species, \textit{Aconitum noveboracense}. In addition, the establishment of tissue culture protocol for the genus \textit{Aconitum} provides a foundation for commercial micropropagation of ornamental aconites, development of new varieties through somaclonal variation, and in vitro cultures for the extraction of valuable alkaloids.

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Literature Cited