

## Propagation of *Cirsium douglasii* and *C. andrewsii* by Tissue Culture for Use as Test Plants in Biological Control of Weeds Research

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### Abstract

Because of limited availability, two indigenous North American plants, *Cirsium andrewsii* and *C. douglasii*, were propagated by tissue culture techniques for use in host-specificity testing of candidate biological control insects. The method of culture from seed sterilization to planting in soil is given.

### Introduction

Micropropagation *in vitro* is mainly used for vegetative propagation of horticulturally-important species, and the list of plants propagated by this method continues to increase as technological advances are made. However, tissue culture techniques for non-agricultural plants are not advanced. The objectives of this research were to: (1) develop a method of producing some of these hard-to-get plants (*Cirsium* spp.; Compositae) *in vitro*; and (2) provide the Biological Control of Weeds Laboratory in Rome with needed plants for their testing program.

To insure that candidate insects used for the biological control of weeds would not damage endangered or native plants if released in North America, it was necessary to include these categories in host-specificity tests. Since the endangered plants and some of the native plants were difficult to obtain in sufficient numbers for testing, micropropagation seemed the tool of choice to produce large numbers, from a very small amount of initial material. The concept of using tissue culture to increase the numbers of individuals of an endangered species was also pursued by Hammett and Evans (1985) in the culture of *Centaurea junoniana* Svent. (Compositae).

### Methods and Materials

#### Propagule Source

Twenty seeds of *Cirsium douglasii* Jepson, collected at Cecilville, California, 6 September 1984, and 20 seeds of *Cirsium andrewsii* Gray collected at Abbott's Lagoon, Point Reyes, Marin Co., California, 12 June 1985, were obtained from the USDA-ARS, Biological Control of Weeds Laboratory at Rome.

#### Seed Treatment

Because seeds were not large enough for coat removal, they were washed in running tap water for 2 h, then immersed in a solution of 200 ppm GA3 (gibberellic acid) for 24 h to help

germination. Next, they were surface sterilized in 10% sodium hypochlorite solution (7% activated chlorine) for 20 mins, and washed three times (5 mins each) in sterile distilled water. Work was performed under a sterile air-flow cabinet.

The sterilized seeds were placed on 10 ml of agar-based proliferation medium in test tubes. After 8 wks 50% of the seeds had germinated, and contamination rate was around 10%.

#### *Medium for Establishment and Proliferation Stage*

The medium consisted of Murashige and Skoog (1962) salts supplemented with 25 mg/l  $\text{NaH}_2\text{PO}_4$ , 20 mg/l  $\text{FeNa}_2\text{EDTA}$ , 100 mg/l myoinositol, 0.4 mg/l thiamine-HCl, 40 mg/l adenine sulphate, 0.1 mg/l 1-naphthalene-acetic acid (NAA), 0.5 mg/l 6-furfurylaminopurine (Kinetin), 0.5 mg/l N-(2-isopentenyl)-adenine (2iP) and 30 g/l sucrose. The pH was adjusted to 5.8, 8 g/l Difco-Bacto agar was added and the medium was autoclaved for 15 mins at 121°C and 1.1 kg/cm<sup>2</sup>. This medium was selected after having set up several experiments with Murashige and Skoog (1962) salts and BA (benzyladenine) at concentrations ranging 0.2 to 1.0 mg/l. The results of using MS and BA were that the proliferation rate was very high, but vitrification was a serious problem. The use of other compounds such as adenine sulphate, kinetin and 2 iP improved the culture vigor and solved the problem of vitrification. These organic compounds are currently used for micropropagation of artichoke (*Cynara scolymus* L.) a thistle in the same tribe (Cardueae) as the *Cirsium* spp.

#### *Culture Conditions*

All cultures were kept in a growth room at  $19 \pm 2^\circ\text{C}$ . and a 16 h photoperiod provided by white fluorescent lights (Philips 33W/86) giving 2000 lux.

#### *Culture Handling*

After one month on the culture medium, some of the non-contaminated seeds germinated and were used as sources of explants. The cotyledons and the initial roots were removed from each explant and the shoot buds were isolated by excising the hypocotyls 0.5 cm below the shoot tips. Each batch of starting material was kept separate and identified by original seed source. The original shoots which had produced axillary shoots were placed into 450 ml-wide mouth jars containing 150 ml of medium, and at 3-wk intervals they were transferred onto fresh proliferation medium. When about 400 microshoots had been obtained, they were transferred to a rooting medium.

#### *Rooting Medium*

Rooting medium was prepared using Tendille and Lecerf's (1974) medium supplemented with 0.01 mg/l biotin, 1.0 mg/l thiamine-HCl, 1.0 mg/l nicotinic acid, 1.0 mg/l D-Ca-pantothenate, 100 mg/l myoinositol, 1.0 mg/l pyridoxine-HCl, 20 mg/l  $\text{FeNa}_2\text{EDTA}$ , 0.5 mg/l 1-naphthalene-acetic acid and 30 g/l sucrose. The pH was adjusted to 5.8 prior to adding 8 g/l Difco-Bacto agar and autoclaving for 15 mins at 121°C and 1.1 kg/cm<sup>2</sup>.

#### *Transfer of Plantlets to Soil*

After an average of 18 d on the rooting medium, the plants were fully rooted. Then they were removed from the medium and any agar still attached to the roots was washed off with tap water. The plantlets were then transplanted to pots (10 cm) with a peat and top soil planting medium (50/50:V/V) and kept under a plastic tent at ambient temperature and high humidity for 3 wks. When they were well established, hardened and growing, the plants were transplanted into 20 cm terracotta pots and moved out-of-doors to mature.

## Results and Discussion

In only a few months we were able to produce about 400 plants of *C. douglasii* and *C. andrewsii*. These results validated micropropagation as effective tool for producing large numbers of native North America plants from a very small amount of initial explant material. Micropropagation could have a significant impact in the vegetative propagation of rare and endangered non-agricultural plants whether for use in host-specificity tests in biological control of weeds research or for augmenting natural populations of these plants.

## References

- Hammett, N. and P.K. Evans. 1985. The *in vitro* propagation of an endangered species *Centaurea junoniana* Svent. (Compositae). *J. Hort. Sci.* **60**:93-7.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**:473-97.
- Tendille, C. and M. Lecerf. 1974. La multiplication vegetative de l'asperge (*Asparagus officinalis* L.). Action de divers facteurs, en particulier de la nutrition minerale, sur le developpement des meristemes et sur la production de plantes adultes. *Ann. Amelior. Plantes* **24**:269-82.