



Propagation of *Trillium* at the Cincinnati Zoo & Botanical Garden

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Abstract

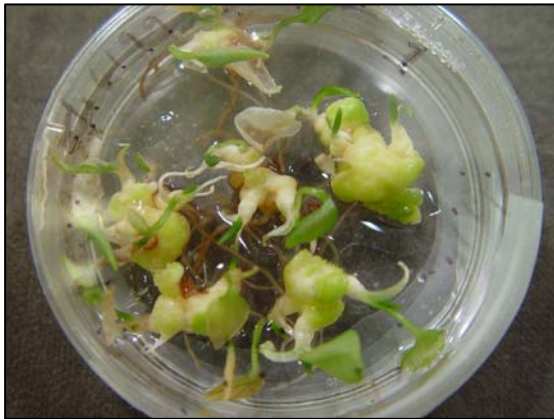
The Plant Research Division of CREW and the Horticulture Department of the Cincinnati Zoo & Botanical Garden are working to apply *in vitro* tissue culture propagation methods to wild and endangered *Trillium* species. Using the procedures for micropropagation combined with those of *in vitro* collecting, research is continuing to evaluate the ability of *in vitro* methods to contribute to the propagation and preservation of *Trillium* species.

In Vitro Propagation Studies

Studies in this laboratory have described aspects of the in vitro propagation of *Trillium* species. Regeneration of plants was accomplished in *T. decipiens*, *T. flexipes*, *T. grandiflorum* and *T. pusillum* var. *pusillum* using immature leaf tissues taken from shoot buds (1). Tissues were surface sterilized with a 1:10 dilution of Clorox for 15 min, rinsed with water and cultured on several media containing Murashige and Skoog (MS) (2) salts plus auxin and cytokinin. In a subsequent study, the responsiveness of leaves and stems was compared in emerging shoots of *T. erectum* and *T. grandiflorum* 4-8 cm in length with the leaves partially expanded but not unfolded (3). The best response with both species was on half-strength MS salts with cytokinin and auxin, from the apical portions of the stem and the basal portions of the leaves, with stems being more responsive than leaves. *T. erectum* was generally more responsive than *T. grandiflorum* under the conditions of this study.

The response of regenerating *Trillium* tissues in vitro is the formation of a swollen white structure, the MR or mini-rhizome. This initially spherical structure enlarges and a small shoot tip emerges, generally from the upper surface. This enlarges, emerging as a leaf. Roots then form from the MR, and once the plant has rooted, it can be transferred to soil for further growth. In the case of *T. decipiens*, plants with the characteristic three-leaved form have been formed within two years.

The procedures developed for more common species have been used to propagate the endangered *T. persistens* (4) and *T. pusillum* var. *texanum*.



Figures, from top to bottom (left to right):

- *Trillium* MRs with shoots
- *Trillium* shoot rooted in vitro
- *Trillium decipiens* propagated from tissue culture



Figures (left to right)

- **Collecting *Trillium* tissues by IVC**
- **Immature seeds of *Trillium luteum***

Current Studies

Current studies are exploring ways of initiating cultures from wild *Trillium* plants with minimal disturbance to the plant. The Plant Research Division at CREW has been a leader in the development of the technique of in vitro collecting, or IVC, in which small pieces of tissue are removed from the plant in the wild, sterilized and put onto sterile medium in the field, leaving the majority of the plant intact (5).

Initial studies in 2007 suggest the challenge of removing tissue that is developmentally young enough for successful culture from wild plants. Tissues removed from stems and leaves that had emerged from the ground, but were still not entirely enlarged, were still too developed to respond well in cultures.

Another approach was tested, using immature seed. Immature seeds from *T. luteum* were surface sterilized and placed onto a minimal medium with no cold treatment. About 8% of these seeds germinated in culture, initiating in vitro cultures of *T. luteum*. This method holds some promise for initiating non-clonal cultures from plants that produce seeds. However, work will also continue to evaluate measures for using IVC on vegetative tissues from wild-grown plants.

References:

1. Pence VC, Soukup VG. 1986. Plant regeneration from *Trillium* spp. in vitro. HortScience 21:1211-1213.
2. Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497.
3. Pence VC, Soukup VG. 1993. Factors affecting the initiation of mini-rhizomes from *Trillium erectum* and *T. grandiflorum* tissues in vitro. Plant Cell Tiss Org Cult 35: 299-235.
4. Pence VC, Soukup VG. 1995. Propagation of the rare *Trillium persistens* in vitro. Bot Gard Microprop News 1: 109-110.
5. Pence VC. 2005. In vitro collecting (IVC) I. The effect of media and collection method on contamination in temperate and tropical collections. In Vitro Cell Dev Biol-Plant. 41: 324-332.

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The Cincinnati Zoo & Botanical Garden

The Cincinnati Zoo & Botanical Garden is one of only two botanical gardens in Ohio accredited by the *American Association of Museums* and is a participating institution in the *Center for Plant Conservation*. As one of the first public display gardens in the United States (1875), the Cincinnati Zoo & Botanical Garden is a leading regional botanical garden with an outstanding plant collection and a unique blend of gardens, landscapes, and exotic animal exhibits.

Center for Conservation and Research of Endangered Wildlife (CREW)

CREW's Plant Research Division uses *in vitro* methods for the propagation and preservation of endangered species, when traditional methods are not adequate. CREW collaborates with partners across the U.S. to use tissue culture methods to help propagate and preserve some of the nation's rarest species. These methods can increase the numbers of plants available for reintroduction, research, preservation, and education. In addition, seeds, spores, and tissues of endangered plants are cryopreserved in liquid nitrogen for long-term storage in CREW's Frozen Garden. These methods can help revive populations and provide a back-up for rare species into the future.

