Chapter 24: Collecting *in vitro* for genetic resources conservation

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Abstract

Since this topic was reviewed in 1995, a technical bulletin as well as some additional reports using *in vitro* collecting (IVC) have been published, but the general needs and applications for IVC, as outlined in 1995, remain the same. When seeds or cuttings are not available to a collector or transport is not practical, tissues collected by *in vitro* methods can provide a valuable tool for obtaining and transporting germplasm. This has been especially useful for the collection of wild, endangered species for propagation for restoration and for tissue banking, particularly for species with recalcitrant seeds or for species making few or no seeds. IVC provides an additional tool for meeting the *ex situ* conservation and restoration goals of Target 8 of the Global Strategy for Plant Conservation.

Introduction

While some additional methods have been demonstrated for use in *in vitro* collecting (IVC), the basic approaches and principles described in 1995 are the same. IVC is a supplemental conservation tool for obtaining plant tissues of both crop and wild species for *in vitro* propagation and preservation. It is especially useful when seed collection is not possible or practical. A technical bulletin, edited by Pence et al. (2002a), provides updates on methods, as well as a number of case studies on crop and some wild species. A few further references since that time provide additional applications of this method, and although initially described for collecting germplasm from crop species and their wild relatives, the technique has been used to collect germplasm from wild endangered species as well (Pence, unpublished data; Pence and Charls 2003; Pence et al. 2009; Trusty et al. 2009). Trials on wild rainforest species have also been reported (Pence, 2005). In all cases, the fundamentals of IVC, as described in 1995, have remained unchanged, including its most prominent characteristic: its flexibility.

This chapter is a synthesis of new knowledge, procedures, best practices and references for collecting plant diversity since the publication of the 1995 volume *Collecting Plant Diversity; Technical Guidelines*, edited by Luigi Guarino, V. Ramanatha Rao and Robert Reid, and published by CAB International on behalf of the International Plant Genetic Resources Institute (IPGRI) (now Bioversity International), the Food and Agriculture Organization of the United Nations (FAO), the World Conservation Union (IUCN) and the United Nations Environment Programme (UNEP). The original text for Chapter 24: Collecting *In Vitro* for Genetic Resources Conservation, authored by L. A. Withers, has been made available online courtesy of CABI. The 2011 update of the Technical Guidelines, edited by L. Guarino, V. Ramanatha Rao and E. Goldberg, has been made available courtesy of Bioversity International.

Current status

In terms of endangered species, IVC remains a valuable part of the conservation toolkit. When courier services are available, rapid and reliable, it is generally more cost effective and equally successful to ship cuttings overnight from the field to the laboratory than to collect by IVC. However, if such service is not readily available, IVC can be used to help maintain viability of the tissue in transit. Also, if the collector is an *in vitro* specialist and needs to remain in the field for further collections, IVC can be useful for holding the tissue, either in the field or after it is sent back to the laboratory, until the collector/tissue culturist can return to the lab to work with it more fully.

One of the first uses of IVC for collecting endangered species was in the U.S. in 1998, for the collection of two rare wetland species: *Lobelia boykinii* and *Rhexia aristosa*. Staff from the labs of the Cincinnati Zoo & Botanical Garden's Center for Conservation and Research of Endangered Wildlife (CREW) invested significant time and resources to conduct a trip to the site of the plants in North Carolina, with the goal of collecting seed from which to initiate *in vitro* cultures for propagation. In collaboration with local field experts, the timing of the trip was determined to correspond to the production of seed, but due to weather and other variables, when the site was reached, the seed had already been dispersed into the surrounding water. However, tools for IVC had also been brought to the site, and shoot tips were removed from several genotypes of each species and cultured in small vials of medium. These were brought back to the laboratory, where they were used successfully to initiate cultures, tissues from which were ultimately banked in long-term liquid nitrogen storage in CREW's CryoBioBank (Clark and Pence 1999 and unpublished). This provided an example of the use of IVC when seeds are not available at the time of collecting. Rare species are often in remote areas, and despite the best information available, the timing of the collecting expedition might not coincide with the availability of seeds. Additionally, unexpected species that are of interest but for which seeds are not available might be encountered during an expedition. Having the option of utilizing IVC methods in these circumstances can maximize the time and resources invested in an expedition.

Recalcitrant seeds, because of their sensitivity to desiccation and often short viability, pose challenges for collecting that are similar to vegetative tissues. If courier services or overnight transport is not available or practical, IVC methods for the seed or embryo can be utilized or vegetative materials can be collected as a back-up to seed material (Berjak and Pammenter 2003).

Even when courier service is available, IVC can be useful for collecting multiple genotypes that must be collected over the course of several days at several sites, decreasing the cost associated with multiple shipments of material. It has been utilized in this way for collecting multiple genotypes of the endangered species, *Asimina tetramera*, from Florida, in the USA. *A. tetramera* has recalcitrant seeds, and CREW has worked with collaborators in Florida to collect multiple genotypes for tissue cryopreservation. These have been collected from multiple sites over the course of several days using IVC, resulting in tissue lines that are being used for producing plants for restoration, as well as for tissues for cryopreservation (Pence and Charls 2003).

One of the crop species for which IVC is routinely used is coconut. This is especially the case in the framework of a research project entitled "Validation of a Coconut Embryo Culture Protocol for the International Exchange of Germplasm", which is funded by the Global Crop Diversity Trust and coordinated by Bioversity International. The project includes coconut research institutes in Brazil, Côte d'Ivoire, Sri Lanka, the Philippines and Papua New Guinea (http://ongoing-research.cgiar.org/factsheets/validation-of-a-coconut-embryo-culture-protocol-for-the-international-exchange-of-germplasm. No new protocols have been published; participants have improved, refined or adapted the existing protocols described in the 1995 version of this chapter to their own needs.

Contamination of initial explants is a challenge for IVC, and its extent will depend upon the species, tissue, environmental conditions and location of the parent plant. A review of some of the approaches to prevent contamination is included in the IPGRI Technical Bulletin (Pence and Sandoval 2002). Tests of antimicrobial agents on non-endangered, wild rainforest species collected in the open air have indicated the usefulness of such methods (Pence 2005). It is particularly helpful if methods can be tailored to specific,

targeted species, as was done in developing IVC methods for species of *Eucalyptus* (Watt et al. 2003). This study showed that methods developed on greenhouse and garden-grown plants could be transferred to species growing in the wild and could provide useful models for developing methods for other species identified as candidates for IVC.

Challenges/needs/gaps

The success of IVC is dependent on the availability of workable methods of *in vitro* culture for any particular species. Less than optimal methods for species that are recalcitrant to culture limit the application of all *in vitro* methods to these species, including IVC. Thus, improvements in the application of tissue-culture methods to a wider range of species will benefit IVC, as well. In addition, although methods for controlling contamination are successful in the majority of cases, improvements in antimicrobial methods should also serve to broaden the applicability of IVC.

Despite its potential, IVC may be viewed as being underutilized as a tool for *ex situ* plant conservation. There may be several contributing factors to this: first, IVC is largely a method for transport. In locations where overnight shipping services are available and reliable, it might be cost-effective to utilize these in order to get the material to a laboratory as quickly as possible. There, the best methods of disinfestation and culture can be applied quickly, without the constraints that might limit those methods in the field. Much of the *in vitro* work with wild species has been reported from areas that have had such shipping services or where the distances did not preclude quick return to the laboratory. Much work with endangered wild species is done in-country, also reducing the distances needed for transport.

In addition, the application of *in vitro* methods for *ex situ* conservation of wild species has been limited, compared with the use of *in vitro* methods for commercial applications. Increased efforts in this area are needed, in order to meet the *ex situ* conservation challenge of the Global Strategy for Plant Conservation. Although many species can be stored by seed banking, there is a subset of species that either have recalcitrant seeds or produce few or no seeds. These exceptional species often require *in vitro* methods for propagation or long-term *ex situ* storage by cryopreservation. IVC could play a significant role in assisting in transporting short-lived seeds, embryos, or tissues, thereby providing materials for *in vitro* propagation and preservation. Further research into the growth of tissues *in vitro* as well as into the technical aspects of IVC would help facilitate the application of these methods to wild endangered plant germplasm.

Conclusions

In vitro collecting has proven to be a workable and useful tool for collecting plant material for *in vitro* propagation and *ex situ* conservation. The needs for IVC are generally unchanged, although the years since 1995 have seen its application broaden to a wider range of species, both cultivated and wild. As conservation efforts increase in the face of continued habitat loss, climate change and other factors, IVC will likely find wider application for collecting plant germplasm for food security and the long-term *ex situ* preservation of endangered species.

Annotated bibliography

NOTE: Several of the references in this list refer to chapters in IPGRI Technical Bulletin No. 7, which is available in PDF format: www.bioversityinternational.org/fileadmin/bioversity/publications/pdfs/866.pdf.

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