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INVITED REVIEW

Biodiversity conservation and conservation biotechnology tools

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Abstract This special issue is dedicated to the *in vitro* tools and methods used to conserve the genetic diversity of rare and threatened plant species from around the world. Species that are on the brink of extinction because of the rapid loss of genetic diversity and habitat come mainly from resource-poor areas of the world and from global biodiversity hotspots and island countries. These species are unique because they are endemic, and only a few small populations or sometimes only a few individuals remain in the wild. Therefore, the challenges to support conservation by *in vitro* measures are many and varied. The editors of this invited issue solicited papers from experts from Asia, Africa, Europe, Australia, and North and South America. This compilation of articles describes the efforts in these diverse regions toward saving plants from extinction, and details the direct application of *in vitro* and cryopreservation methods. In addition, these contributions provide guidance on propagation of rare plants, including techniques for large-scale propagation, storage, and reintroduction. The *in vitro* techniques for conserving plant biodiversity include shoot apical or axillary-meristem-based micropropagation, somatic embryogenesis, cell culture technologies and embryo rescue techniques, as well as a range of *in vitro* cold storage and cryopreservation protocols, and they are discussed in depth in this issue.

Keywords Global biodiversity hotspots · Conservation · Cryopreservation · Micropropagation

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Introduction

Worldwide, many plant species are threatened with extinction because of the gradual disappearance of the terrestrial natural ecosystems for various human activities. Often, this is due to the clearing of indigenous vegetation for agriculture and the resulting erosion, salinization, and invasion of alien species, but more recently climate change is looming as a significant new threat. More than 50% of the world's plant species are endemic to 34 global biodiversity hotspots (GBH), which once covered 15.7% of the earth's land surface and which are now reduced to 2.3%. These areas include large numbers of endemic species, which face an increasing threat of extinction, The International Union for Conservation of Nature Red List of Threatened Plants, first published in 1998 (IUCN 1998), lists more than 8,000 species currently (IUCN 2010). *In situ* conservation alone is not sufficient to meet the challenges of saving endangered species. While seed banking can be utilized for *ex situ* conservation of the majority of endangered species, there are a significant number of species for which seed banking is not an option. Conserving the genetic diversity of species and populations is highly important in these situations, and micropropagation can provide large numbers of propagules from a cross-section of the genetic diversity of a region (Rogers 2003).

Initiatives developed and directed by international agencies for the integrated conservation of rare and threatened plant species both *in situ* and *ex situ* are needed. Botanical gardens are increasingly charged with collecting and maintaining endangered species, although funding is often scarce. The Global Strategy for Plant Conservation (GSPC 2010), signed by more than 180 countries in 2002, aims to stem this loss by setting targets for understanding and conserving plant diversity, promoting sustainable use, providing education and building capacity to support plant conservation. Target 8 of the GSPC, updated for 2020 in Nagoya, Japan, in 2010, calls for "at least 75% of threatened plant species in *ex situ* collections...and at least 20% available for recovery and restoration programmes" as noted by the United Nations Development Program (UNDP 2010a). Another initiative developed by the United Nations member states and more than 20 international organizations is that of The Millennium Development Goals (MDGs; UNDP 2010b). Of the eight MDG targets, Target 7 Goal e (UNDP 2010a) is aimed at significantly reducing the rate of loss of biodiversity by 2015.

While plant conservation is progressing through the use of *in situ* protection and *ex situ* preservation in botanical gardens and seed banks, additional approaches are needed for some of the most endangered species. Complementary strategies of *in situ* conservation combined with *ex situ* seed, *in vitro* and cryopreserved storage can be important for preserving the biodiversity of many endangered habitats.

In vitro tools

In vitro seed germination, vegetative propagation, and acclimatization methods contribute to propagule production, both for cryopreservation and for translocation or reintroduction projects. In addition, *in vitro* culture techniques are being used to study the growth habits of plants, as well as the ecological factors that influence their growth and development (*i.e.*, *in vitro* ecology). Although standard *in vitro* propagation methods are available, endangered species may have unusual growth requirements and thus may require modified procedures for *in vitro* culture. In addition, the limited amount of plant material available from rare species poses major challenges in the application of *in vitro* methods to endangered species (Sarasan *et al.* 2006).

In vitro methods provide tools that can be used in a variety of ways, depending on the need of the species. Collecting cuttings of plants and seeds is generally the most cost-effective method for providing material for *ex situ* conservation. However, there are many instances in which seeds are sterile or not available, or so few plants remain that collecting whole plants would negatively impact the population. *In vitro* collecting of tissues is less invasive than removing whole plants and allows for an efficient sampling of a large number of plants when seeds are not available.

In addition, many tropical species have seeds that die when dried or frozen (recalcitrant seeds), and thus they cannot be stored using conventional seed-banking technologies (Chin 1996). Established cultures can comprise a medium-term *ex situ* collection, but these cultures can also provide tissues for cryopreservation and long-term storage. The importance of *in vitro* culture for medium-term (1–5 yr) and cryopreservation for long-term conservation (>5 yr) is well established (Ashmore 1997; Reed *et al.* 2005; Reed 2008). *In vitro* techniques provide the option of cryopreserving embryos or vegetative tissues for long-term storage as an alternative to seed banking, for a number of tropical timber and fruit resources. Providing long-term storage for many of these recalcitrant seed species is especially challenging, as they do not respond well to traditional *in vitro* or cryopreservation protocols, thus requiring new approaches to tackle these problems (Normah and Makeen 2008).

The use of *in vitro*-propagated plants for reintroduction or restoration of rare species is also finding application, and this relies on the development of successful methods for acclimatizing plants from culture to *in situ* conditions. Further, as we learn more about the metabolic requirements of diverse plants, design of better growing conditions and nutrient media to fit the specific needs of rare plants should ease the difficulty of growing threatened and endangered species. The range of *in vitro* tools available for plant conservation is highlighted in this issue by Engelmann. In addition, Kaczmarczyk *et al.* discuss the need to focus on new technologies for cryopreservation and long-term storage of rare and endangered plants.

The need for *in vitro* tools

Biodiversity hotspots around the globe are at risk, and *in vitro* and cryopreservation research on endangered plants is in progress in many countries, including in Australia (Ashmore *et al*. 2011), Malaysia (Normah *et al*. 2008) and South Africa (Berjak *et al*. 2011). This issue highlights these ongoing efforts and the need for additional attention to neglected and endangered tropical plant species. Unfortunately, compared to animal conservation, plant conservation is not adequately supported worldwide (Pennisi 2010).

Logging in tropical biodiversity hotspot areas to provide timber and land for crops such as rubber, sugarcane and oil palm is on the increase, and this is contributing to massive losses of biodiversity (*e.g.*, Amazon basin; Papua, New Guinea; Indonesian forests). Complimentary conservation strategies of *in situ* seed storage and *in vitro* or cryopreserved storage are important to many endangered habitats, such as those found in Brazil and noted in this issue (Pilatti *et al*. 2011). Temperate forests also require protection, both for lumber species as well as for the diverse endemic members of these ecosystems (Pijut, 2011). Biodiversity hotspots in temperate and sub-tropical zones are less publicized than their tropical counterparts, but they similarly contain many threatened and endangered plants. These hotspots are being addressed with a range of *in vitro* conservation techniques in projects on several continents noted in this issue (Ashmore *et al*. 2011; Krishnan *et al*. 2011; Martín and González-Benito 2011; Munoz-Concha and Davey 2011). Conservation of lower plants is considered a priority subject worldwide, and *in vitro* conservation of European bryophytes and endangered ferns is used to complement *in situ* activities (Rowntree *et al*. 2011; Barnicoat *et al*. 2011).

More than 50,000 species are used globally as sources of medicine, food, cleaning, personal care and perfumery, and these are collectively known as medicinal and aromatic plants. Such species are often collected illegally or unsustainably from the wild and, as a result, may lose genetic diversity and face the threat of extinction. Improving propagation and availability of these plants could protect those remaining in the wild and provide revenue for resource-poor areas. *In vitro* methods can often be the most efficient way to clonally propagate a species without depleting wild resources and can be an important part of an integrated program for the conservation of medicinal and aromatic plants worldwide. A description of *in vitro* techniques used in one biodiversity hotspot, the tropical and subtropical Western Ghats region of India, shows how these techniques are used to conserve medicinal plants of this region of biodiversity (Krishnan *et al*., 2011).

Orchids, one of the largest and most diverse families of flowering plants, face an uncertain future through unscrupulous collection, the impacts of climate change and habitat loss (Swarts and Dixon 2009). The complex requirements for pollination, seed germination, and many other biotic and abiotic factors contribute to loss of plants in the wild when these requirements are not met. *In vitro* orchid seed germination, cryopreservation and reintroduction studies are discussed in this issue in the context of Australian and US orchid conservation projects (Ashmore *et al*. 2011; Kauth *et al*. 2011). These studies shed light on the need for more work on orchid conservation, especially those species that are terrestrial and require more complex interactions with mycorrhizal partners. More detailed study to understand mycorrhizal associations in terrestrial orchids is urgently needed to improve *in vitro* orchid seed germination for recovery, storage and restoration activities. Likewise, a major limitation of current orchid conservation efforts has been the focus on single aspects of orchid conservation biology (*i.e.*, propagation, pollination biology, *etc*.). Orchid conservation practices must integrate an understanding of existing and future threats, population fluctuation, pollination and reproductive biology, *in vitro* and *in situ* propagation, and maintenance of population genetic diversity as tools in species conservation and recovery. Interest is growing worldwide in restoration ecology projects that lead to species or ecosystem conservation. Reintroduction and translocation projects aimed at the restoration of damaged ecosystems all need genetically diverse, high quality propagules.

Conclusions

For species threatened with extinction, *in situ* conservation may be the best option; however, this alone may not always be sufficient to insure the survival of a species. In addition to standard methods such as seed banking and development of living collections, *in vitro* tools can provide additional backup collections and provide alternative propagation and conservation of species. Seeds or vegetative material from genetically diverse wild populations can be difficult to obtain, and methods should be in place for the propagation, storage, sustainable utilization, and storage of these genetic resources in liquid nitrogen. *In vitro* techniques are well developed for the collection, propagation and cryopreservation of many species. In addition, the tools of molecular biology and molecular genetics can aid in the development of more effective propagation and storage technologies. As extinction pressures are increasing, it is important that priority species are identified and that integrated conservation measures are undertaken, utilizing all the tools available, including the *in vitro* methods that are the subject of this volume.

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