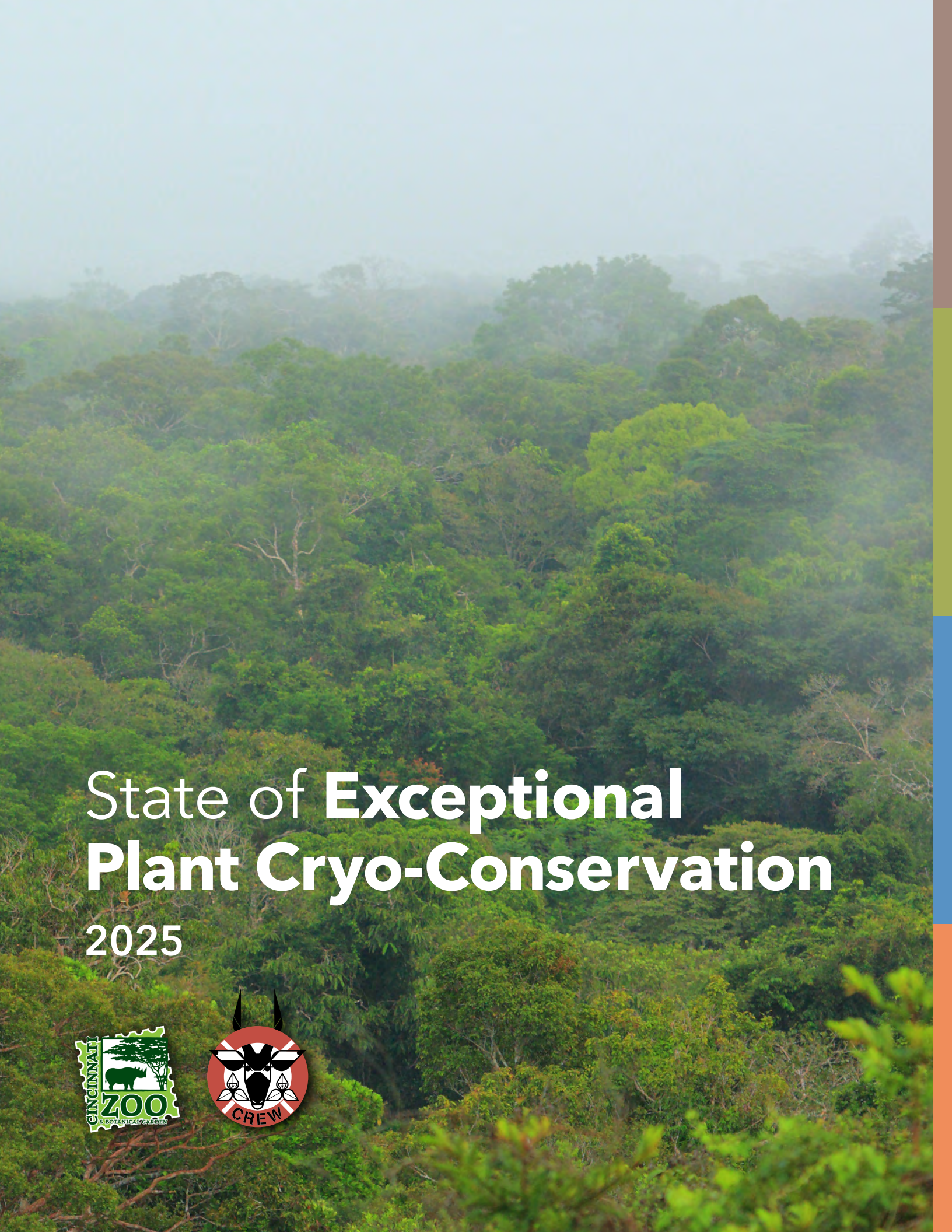

State of **Exceptional Plant Cryo-Conservation**

2025

CINCINNATI ZOO & BOTANICAL GARDEN

CREW

State of Exceptional Plant Cryo-Conservation

2025

The Center for Conservation and Research of Endangered Wildlife (CREW) of the Cincinnati Zoo & Botanical Garden has the mission of Saving Species with Science®. Its Exceptional Plant Signature Project uses research, propagation, cryopreservation, ex situ conservation in the Frozen Garden® of CREW's CryoBioBank®, genetics, restoration, and education to preserve endangered plants, particularly those that cannot be conserved using conventional seed banking. The Exceptional Plant Conservation Network (http://cincinnatizoo.org/epcn) is working to provide resources and connect researchers to facilitate the conservation of exceptional plants worldwide.

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Cover Photo: Brian Jorg
Design: Toree Bodner, Graphic Designer, Cincinnati Zoo & Botanical Garden

State of Exceptional Plant Cryo-Conservation 2025

Table of Contents

Executive Summary

It's been stated that there is no technical reason for any plant species to go extinct, given the current knowledge and multiple options for facilitating both in situ and ex situ conservation (Smith, 2016). Indeed, efforts directed at both in situ and ex situ conservation are increasing, protecting habitats and creating extensive ex situ living collections and large seed banking efforts (Linsky et al., 2024; Liu et al., 2020). However, the projections are that the trajectory of species extinction will continue to rise (Antonelli et al., 2023), and the question becomes how quickly and effectively can conservation actions be applied to prevent extinction losses (Anderson et al., 2025)? For those species that require technologies beyond seed banking—the exceptional species—there is an additional layer of challenge.

This document provides a summary of the current state of exceptional plant conservation, specifically cryo-conservation, based on the *2nd Virtual Global Symposium and Workshop on Conserving Exceptional Plants: Cryobiotechnologies, Status, and Scale*, held April 8–9, 2025, and hosted by the Cincinnati Zoo & Botanical Garden's Center for Conservation and Research of Endangered Wildlife (CREW). It provides a snapshot of the current state of exceptional plant conservation cryo-banking, looking at the current methodologies, global capacity, technological challenges and opportunities that will be needed for scaling up efforts, and finally, evaluating the status, setting priorities, and filling gaps. For this document, the speakers from the symposium have provided short topical summaries of their talks, and each is followed by a link to the full talk.

Section I provides updates on current cryopreservation methodologies, organized around the tissue types that are most frequently used for cryo-banking. While short-lived seeds appear to be the least challenging, determining their short-lived status is less than straightforward. Pollen and spores have characteristics analogous to seeds and should be integrated into long-term ex situ conservation strategies, while for winter-hardy woody species, cryopreserving dormant buds can be an efficient and useful approach. Shoot tip and somatic embryo cryopreservation require additional protocols for developing in vitro cultures and methods for regrowing plants, but they can be useful when other methods are not workable. By matching the most efficient but effective approach to the appropriate tissue, these methods provide a versatile toolbox for approaching cryo-conservation.

However, even with effective methods, cryo-conservation of exceptional species will only be achieved through the efforts of multiple labs and researchers across the globe. **Section II** provides examples of labs around the world that have cryopreservation capacity and are using it for conserving exceptional species, including labs from Australia, Thailand, China, India, South Africa, Europe, Brazil, Mexico, and the United States. While only a snapshot of the full worldwide capacity, these examples provide insight into the different approaches and challenges found globally. In some cases, there is a high level of capacity and expertise, but their application is often heavily weighted toward crops in comparison to wild species.

One point of agreement among plant cryopreservation practitioners is that methods for developing and applying protocols to wild species are time and resource intensive and with increasing threats to plant biodiversity, current practice may not keep pace with extinction. **Section III** presents examples of research that is working toward improving efficiencies that can help scale up cryo-conservation, from a key for identifying likely recalcitrant seeds, to methods for triaging seeds suspected as being short-lived, and approaches for decreasing the time and resources for in vitro work and cryopreservation protocol development. These talks highlight the need for additional, comparative studies to better understand the physiological mechanisms that underlie seed storage characteristics and allow or inhibit survival of tissues in vitro and through cryopreservation. Without this knowledge, scaling up cryo-conservation to the levels needed will not be achieved.

Section IV focuses on moving forward in exceptional species conservation. These talks describe current knowledge of which species are exceptional and its gaps, a framework for developing strategies for conserving exceptional plants, and the Global Plant Cryopreservation Initiative, a "long-term model" for cryo-conservation, focused around three crop species. Planning for exceptional plant cryo-conservation is very feasible, but it will require additional funding and capacity to achieve its goal.

These summaries and talks demonstrate that there is a mature scientific and technological foundation for conserving exceptional plants using cryobiotechnologies that forms the basis for the affirmation that there is no technological reason for any species to go extinct. There is a growing global network of laboratories with cryopreservation capacity that have an increasing awareness and eagerness to apply their expertise to exceptional plant conservation. While there is still a need for additional capacity, training, and focus on wild species, this increasing awareness is a hopeful sign. Information gaps still exist and additional research is needed to streamline the process of protocol development if the needed efficiencies are to be achieved, but new approaches for identifying likely exceptional species, for prioritizing target species, and for streamlining protocol development show promise. Additional support for these efforts should also develop from emerging information databases on in vitro and cryopreservation work and from AI and robotics technologies. What is needed is an overarching global strategy for harnessing these tools and scaling up efforts, as well as investments to support such work. We hope that this overview of the current state of exceptional plant cryo-conservation can help provide context as well as inspiration for both, to ensure that the conservation of exceptional plants is not left behind, and that the application of current and emerging knowledge can outpace the extinction crisis of the 21st century.

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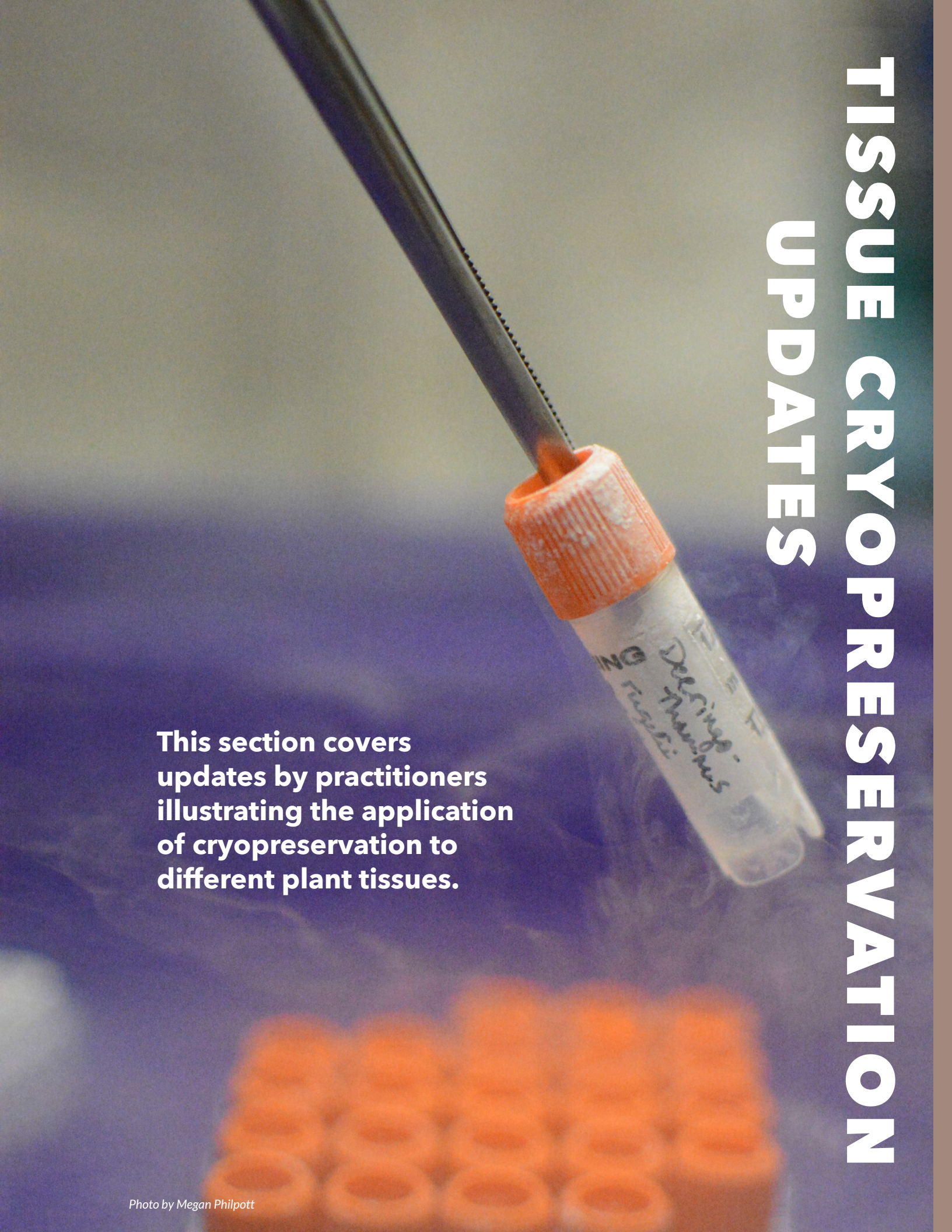
TISSUE CRYOPRESERVATION UPDATES



This section covers updates by practitioners illustrating the application of cryopreservation to different plant tissues.

Photo by Megan Philpott

Cryopreservation of **Short-lived Seeds**

Louise Colville

Royal Botanic Gardens, Kew, United Kingdom

More than 2.4 billion seeds from around 40,000 wild plant species are conserved at Royal Botanic Gardens, Kew's Millennium Seed Bank (MSB) in the UK. The seeds are stored in hermetically sealed containers at 15% relative humidity (RH) and -20°C. These conditions are suitable for the majority of seed-bearing plants that produce desiccation tolerant (orthodox) seeds. Under these conditions, seeds are expected to survive for decades or even centuries, and for around 80% of seed accessions conserved at the MSB this appears to be the case. However, for the remaining 20% there are signs that the seeds may not be quite so long-lived, and significant decline in germination has been detected during routine germination re-testing at 10-year intervals. For this reason, since 2012 the MSB has been duplicating short-lived seed accessions to cryostorage, where up to half of an accession is stored in the vapour phase of liquid nitrogen (c. -180°C).

There are several challenges with identifying species with short-lived seeds based on germination re-test data, including:

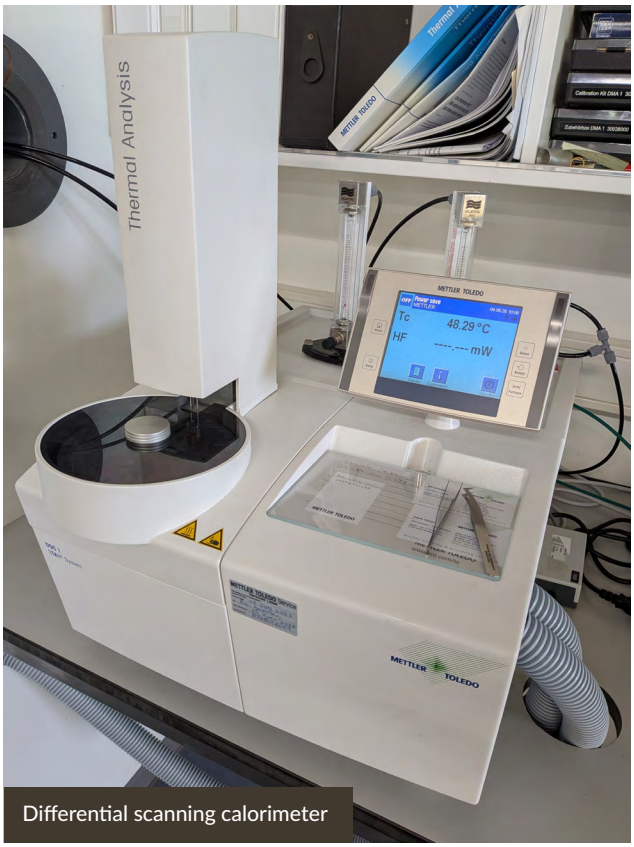
I. The small sample size used for germination tests

II. The use of sub-optimal germination conditions or dormancy-breaking treatments can result in spurious low germination results

III. Most seed collections are too young to detect changes in viability—there is not enough data to inform decision making

IV. Many species are represented by just one accession, but where there are multiple accessions, intra-specific variation is very high due to the effects of maternal environment and post-harvest handling

Furthermore, whilst reducing the storage temperature from -20°C to c. -180°C is expected to be beneficial for seed lifespan (Nadarajan et al., 2023),



Differential scanning calorimeter

the effects of cryostorage on orthodox seeds is not yet fully understood, and there is limited real-time storage data to compare longevity under conventional seed bank storage with cryostorage (Ballesteros et al., 2020). In addition, duplicating accessions to cryostorage once they start to show a decline in germination will likely be less effective for extending their lifespan than if they had been stored at ultra-low temperature from the start (Walters et al., 2004).

> **"More than 2.4 billion seeds from around 40,000 wild plant species** are conserved at Royal Botanic Gardens, Kew."

Research into the basis for seed longevity is seeking to develop tools to predict and identify species with

short seed lifespan. One approach that shows promise is the use of differential scanning calorimetry (DSC) to screen the thermal properties of seeds, particularly for oily seeds in which the melting and crystallisation of the lipids can indicate poor storability at -20°C (Sommerville and Offord, 2022). To date seeds of more than 200 wild species conserved at the MSB have been screened using DSC, and some associations between short seed lifespan and lipid thermal profiles have been identified, including lipid melting and crystallisation events occurring across a wide temperature range and spanning the storage temperature of -20°C (Mira et al., 2019). Lipid thermal fingerprints generated using DSC could be a useful tool for predicting poor storage performance of oily seeds, and enable alternative storage approaches, such as cryopreservation, to be implemented from the outset to improve the longevity of oily seeds in *ex situ* conservation.

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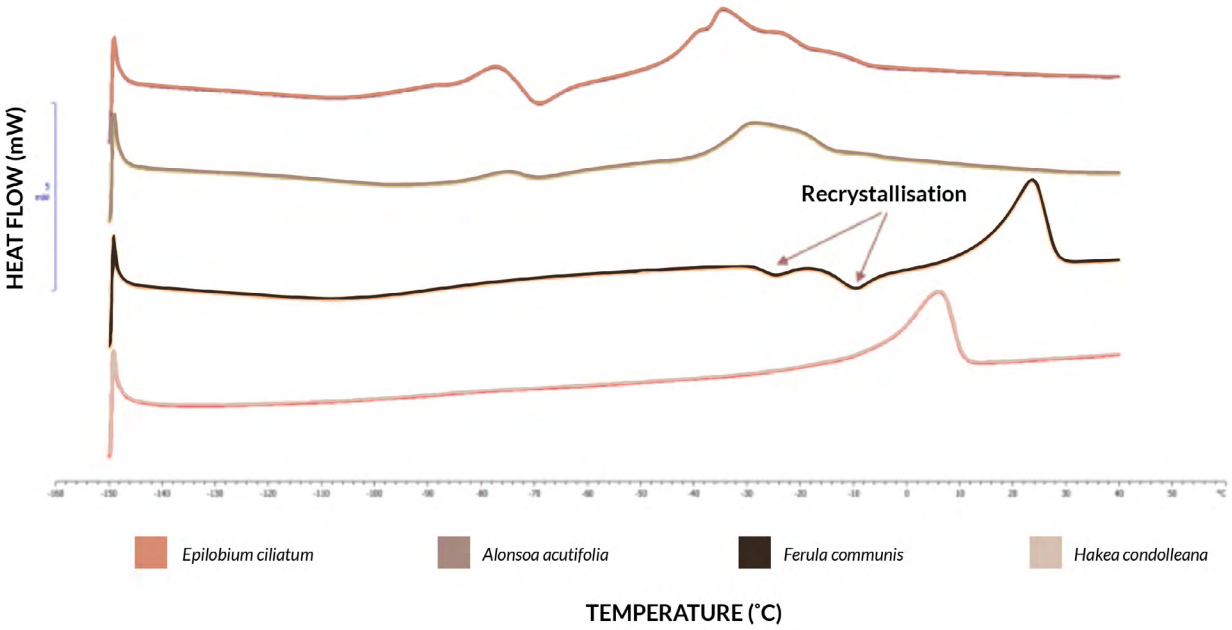


Figure 1. *DSC thermograms showing variation in lipid melting properties in seeds of four species*

Preserving **Embryos and Embryonic Axes**

Karen Sommerville

Botanic Gardens of Sydney, Australia

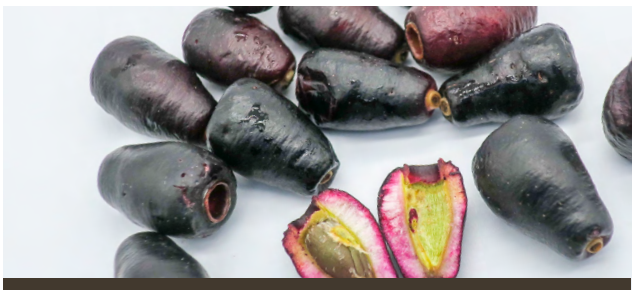
Cryopreservation of embryos and embryonic axes (EAs) is a good option for preserving species with large or oily, non-orthodox seeds (Reed et al. 2008). Embryos and EAs can be dried, frozen and thawed more quickly than the whole seeds they are extracted from and that can greatly improve their regeneration potential following cryopreservation. Another advantage of the method is that it allows the regeneration of an entire plant without the complex tissue culture protocols required for shoot tips.

The main steps involved in the cryopreservation of embryos and EAs are:

1. careful excision of the embryo/EA from the whole seed
2. physical or chemical desiccation to reduce moisture content
3. rapid cooling
4. rapid re-warming after storage
5. recovery on a suitable medium.

Each of these steps may need to be optimised for best results. Specific protocols for individual species are available in Normah & Makeen (2008) and in a variety of papers cited in Nagel et al. (2024).

One of the most important components of developing a protocol for any given species is working out how to excise the embryo/EA without damaging it and then checking whether it will still produce a normal seedling. The recovery medium used will need to replace the nutrients normally supplied by the endosperm or cotyledons, so research to identify suitable medium components may be needed. For *Macadamia integrifolia* cv 'Beaumont,' for example, normal seedling growth was only achieved when a small amount of cotyledon remained attached to the EA and the recovery medium (modified MS) contained activated charcoal and GA3.



Syzygium fullagarii, fruit and seed. Photo by Karen Sommerville

The reduction of moisture content can be achieved by drying the material at ambient humidity, over silica gel, in the air stream of a laminar flow cabinet, or by flash drying or exposure to high sucrose medium. Many intermediate species have been successfully dried over silica gel or in a laminar flow cabinet (Nagel et al. 2024). Embryos and EAs from recalcitrant species seem to do better with flash drying and may need to be desiccated on high sucrose medium before applying cryoprotectants.

The cooling and re-warming steps of the cryopreservation process are best done rapidly. The most common method for cooling involves placing the material in cryovials or foil packets and immersing them directly in liquid nitrogen. Re-warming is often carried out by placing the container in a water bath at 37 to 45°C for a few minutes. The final check for survival and regeneration is dependent on having identified a recovery medium that supports normal seedling development.

Cryopreservation of embryos or EAs has been investigated for more than 125 non-orthodox species over the past 40 years. Regeneration percentages of 90-100% have been achieved for intermediate species like *Corylus americana*, *Juglans nigra* and *Pistacia vera* from temperate zones, and for *Coffea arabica*, *Passiflora edulis* and *Citrus* species from tropical zones. Recalcitrant species have proven more difficult to conserve, but more than 30% regeneration has been achieved for around half of the recalcitrant species tested to date (Nagel et al. 2024).

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Spore and Pollen
Cryopreservation

Daniel Ballesteros

Botanical Garden of the University of Valencia, Spain

Royal Botanic Gardens, Kew, United Kingdom

Spores and pollen are haploid reproductive units of land plants that play a fundamental role in dispersal, survival, and genetic continuity. Despite structural and compositional differences between bryophyte spores, pteridophyte spores, and pollen, many share important physiological properties, such as tolerance to desiccation and low temperatures. These properties facilitate low-tech long-term storage under both conventional seed storage and cryogenic conditions, making them suitable targets for ex situ conservation strategies.

Longevity varies widely across and within structures. For example, chlorophyllous spores (from both bryophytes and ferns) and trinucleate pollen exhibit short lifespans, requiring rapid processing and cryopreservation. On the other hand, non-chlorophyllous pteridophyte spores and binucleate pollen often show greater desiccation tolerance and extended viability. Nonetheless, pollen tend to present generally shorter life spans than spores and diverse non-chlorophyllous fern spores show longevity issues at -20°C due to lipid crystallization, supporting the cryogenic storage of these materials too.

Spore and pollen banking, though less developed than seed banking, represents a promising tool for preserving plant biodiversity. Pollen banking is particularly helpful to preserve male genotypes for taxa with recalcitrant seeds or exceptional germplasm. Established protocols involve careful collection, drying under controlled relative humidity (ca. 30% relative humidity), and storage at temperatures ranging from refrigeration to liquid nitrogen, depending on the longevity needs of the materials banked. Active spore and pollen banks in America, Europe, Asia and Australia demonstrate the feasibility of this approach, yet global coverage remains limited. For example, there are less than ten fern spore banks currently in operation, and, to our knowledge, there are no bryophyte spore banks in operation. Evidence from recent guidelines confirms that mature, properly dried spores and pollen can be conserved under standard seed bank protocols, although cryogenic storage is advised. By integrating spores and pollen into germplasm conservation networks, high genetic diversity can be safeguarded in a cost-effective and space-efficient manner, complementing traditional seed banks and supporting long-term biodiversity conservation.

> "By integrating spores and pollen into germplasm conservation networks, **high genetic diversity can be safeguarded in a cost-effective and space-efficient manner."**

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Dormant Bud
Cryopreservation

Gayle M. Volk

Colorado State University
(affiliate faculty)
USDA-ARS (retired), United States

Cryopreserving nodal sections of woody dormant buds is a cost-effective method to conserve some plant genera that are winter hardy and growing in cold-hardy locations. Dormant bud cryopreservation has been implemented within ex situ genebank settings for decades, although it is still relatively limited in scope compared to the number of genera cryopreserved as shoot tips. Genera that have been cryopreserved as dormant buds include *Malus*, *Prunus*, *Pyrus*, *Fraxinus*, *Salix*, *Diospyros*, *Morus*, *Rubus*, *Ribes*, *Vaccinium*, and some species of *Juglans*. The success of the procedure may vary with winter conditions, species, and for individuals within species, thus requiring adequate preliminary testing before the methods are applied to a wide range of materials (Tanner et al., 2021).

When successful, dormant bud cryopreservation has a significant advantage over shoot tip cryopreservation because a single technician can cryopreserve over 100 accessions in a single winter season. Here, the cryopreservation process for *Malus* dormant buds is provided as an example (Volk et al., 2020). Twigs containing dormant buds are harvested in the peak of the winter season (January in North America), ideally after several days of temperatures below 0°C. The branches containing dormant buds are kept cold (ideally about -3°C) until they are cut into segments containing one or two nodes (35 mm single node sections for *Malus).* Nodal sections are then dried at -3°C to a target moisture content of 25-30% (fresh weight basis). The nodal sections are then packaged into polyolefin tubes (10 per tube), sealed, and then slow-cooled to -30°C at -1°C/h in an electric programmable cooler and held at -30°C for 24 h. The tubes containing dormant bud segments are placed into the vapor phase of liquid nitrogen (LNV) for long-term storage. A subset (usually one or two tubes) of the cryopreserved dormant buds is retrieved from LNV for viability assessments. The tubes are held at 2 to 4°C and then rehydrated in moist peat moss and grafted onto actively growing seedling rootstocks, with two buds per rootstock. Data are collected after 8 to 10 weeks, when the new branches have elongated and produce leaves. In some cases, grafting is not possible, and the buds on the dormant bud segments can be sprouted (Tanner et al., 2020). Sprouted buds can then be surface sterilized and introduced into in vitro culture to produce viable plants. The procedure is demonstrated in an e-book chapter by Volk et al. (2020).

Figure 1. *Cryopreservation of Malus dormant buds. Nodal sections (top, G. Volk), cryopreserved polyolefin tubes in the vapor phase of liquid nitrogen (middle, G. Volk), and sprouted bud after grafting (bottom, R. Bonnart).*

Click Here for References

For More on Dormant Bud Cryopreservation From Dr. Volk and Colleagues, Click Here.

Shoot-tip
Cryopreservation

Raquel Folgado

The Huntington Library, Art Museum, and Botanical Gardens, United States

Cryobiotechnologies combine micropropagation and cryopreservation and can be used for conserving plants with non-orthodox seeds and those requiring vegetative propagation. Shoot-tip cryopreservation, specifically, cannot be implemented without a robust micropropagation protocol that provides effective regeneration after long-term storage at the ultra-low temperature of liquid nitrogen (-196°C).

To successfully preserve shoot tips through cryopreservation, intracellular ice formation, which can irreversibly damage cells during freezing, must be prevented. Shoot-tip cryopreservation methods achieve this through slow cooling, encapsulation-dehydration, or vitrification-based methods.

Slow cooling uses two-step cooling with a cryoprotectant solution to induce freezing dehydration, achieved by gradually lowering the temperature to -30 °C or lower, during which water is pulled from the cells. This is followed by a rapid immersion in liquid nitrogen.

The encapsulation-dehydration technique uses calcium alginate to encapsulate and protect the tissues. After encapsulation, the tissues are subjected to a pre-culture in sucrose, then desiccated under sterile airflow at room temperature or with silica gel, and subsequently either cooled or directly transferred to liquid nitrogen.

Vitrification-based techniques employ more concentrated cryoprotectant solutions and rapid cooling via direct immersion into liquid nitrogen. Rewarming is also rapid, and the potentially toxic vitrification solution is removed. One form of this procedure is droplet vitrification, currently the most widely used technique for cryopreserving shoot tips. The process begins with exposure to vitrification solutions in two steps: loading and vitrification. The sample is then placed on a drop of cryoprotectant on aluminum foil and submerged directly into liquid nitrogen.

While droplet vitrification can be very effective, cryopreserving shoot tips still poses challenges. Currently, there is no universal protocol applicable to shoot tips across all plant species, and recovery rates after cryopreservation vary by cultivar and species. As a result, protocol development may be necessary for individual species or genotypes, optimizing one or more steps in the process. Examples from our lab illustrate this.

For shoot tips of *Aloe veseyi* and *A. fievetii*, plant recovery after cryopreservation improved when donor shoots were exposed to osmotic stress via a high-sucrose medium and when antioxidants were administered before and after cryostorage. For both *Magnolia ashei* and *Persea americana*, chilling temperatures enhance shoot tip regeneration after cryopreservation. For *Persea*, sucrose pretreatment also improves recovery in specific cultivars.

When developing a shoot-tip cryopreservation protocol for a new species, the first step is to search for protocols that have been successful with closely related species, if available. It is also important to consider any information on tolerance to abiotic stresses that species may exhibit. Choosing droplet vitrification as a starting point and using antioxidants can also be beneficial. It is also crucial to closely pay attention to the dissection of shoot tips, as tip size and tissue wounding can influence survival after cryopreservation.

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Advances and Challenges in the Cryopreservation of **Embryogenic Cultures in Woody Species**

Mª Teresa Martínez & Elena Corredoira

Unidad Técnica Biotecnología y Mejora Forestal, Misión Biológica de Galicia (MBG-CSIC), Spain

Somatic embryogenesis is widely regarded as one of the most fascinating demonstrations of plant cell totipotency and serves as a key tool for the propagation and conservation of woody species. Cryopreservation—the storage of embryogenic cells or somatic embryos in liquid nitrogen (LN) at -196°C—offers a reliable method for long-term preservation without compromising their embryogenic potential. This procedure not only facilitates the management of embryogenic lines by reducing the time and costs of their maintenance, but also minimizes the risks of somaclonal variation and contamination. When embryogenic cultures are available, cryopreservation of somatic embryos becomes the preferred approach for the long-term storage of woody plant material. Although the technique has proven technically feasible in over 50 woody species, its successful application still requires careful optimization. Key factors include selecting the appropriate explant type and determining the most effective cryoprotective treatments to ensure consistent post-thaw recovery and regeneration. Our experience shows that the developmental stage of the somatic embryos is critical to post-thaw recovery. The most suitable explants include proembryogenic masses (PEMs) in conifers, early-stage somatic embryo clusters in fruit and deciduous species, and polyembryoids in palms. Early embryogenic stages are generally more tolerant to cryogenic storage in LN than advanced stages like cotyledonary embryos, likely due to their lower levels of vacuolization and cellular differentiation. These traits directly influence the capacity for successful regeneration. In terms of methodology, the classical slow-cooling technique—preceded by cryoprotection with dimethyl sulfoxide (DMSO) and sugars such as sorbitol or sucrose—is most widely used in conifers. In contrast, vitrification-based methods, particularly vitrification and droplet-vitrification using PVS2 solution and rapid LN immersion, have proven more effective in deciduous and fruit species. Despite notable advancements, several critical challenges continue to hinder large-scale biodiversity cryoconservation. These include: (1) the difficulty of inducing somatic embryogenesis in many woody species; (2) the absence of standardized protocols across taxa; (3) the need for reliable methods targeting more advanced embryonic stages; and (4) the challenge of scaling existing techniques to meet conservation demands. While cryopreservation techniques are technically well established for numerous species, their practical application in operational cryobanks remains limited. With few exceptions—such as certain conifers, cork oak, and oil palm—most protocols have been tested on a narrow range of genotypes. This highlights the urgent need for inter-laboratory validation and broader testing across diverse genetic backgrounds. Progress in the field will depend on the development of simplified, scalable protocols that minimize reliance on toxic cryoprotectants. Additionally, collecting and sharing long-term field performance data will be essential to evaluate the agronomic and ecological value of cryopreserved materials, especially in long-lived tree species.

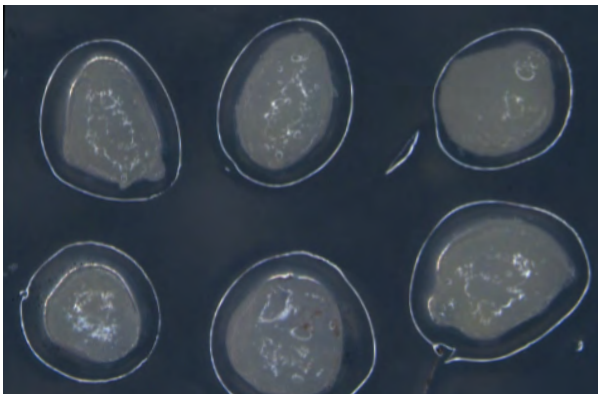

Figure 1. *Globular embryos of Quercus ilex used as explants for cryopreservation.*

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GLOBAL UPDATES

This section covers a selection of labs that provide insights into cryopreservation practices and areas of focus from different parts of the world.

Photo by Brian Jorg

Australia
State of Exceptional Plant Cryo-Conservation

Bryn Funnekotter

Kings Park Science, Department of Biodiversity, Conservation and Attractions, Kings Park WA 6005, Australia

Curtin School of Diagnostic and Therapeutic Sciences, Curtin University, Australia

Australia is home to more than 21,000 plant species, with 1,482 listed as threatened and in need of conservation (DCCEEW 2025). Martyn Yenson, Sommerville et al. (2023) outlined a framework to identify, classify and outline potential ex-situ conservation approaches for exceptional species found in Australia. This study identified over 249 exceptional species with suggested ex-situ conservation approaches including living collections, tissue culture, cryopreservation and seed production areas to compliment conventional seed banking (Martyn Yenson, Sommerville et al. 2023). While this publication focused on threatened species, many more common species found around Australia are expected to be classified as exceptional as research continues assessing seed collections to identify species with recalcitrant seed, deeply dormant seed or that are short lived under standard seed banking conditions (Sommerville, Errington et al. 2021, Balasupramaniyam, Merritt et al. 2025). A family of increased focus in Australia has been the Myrtaceae due to the recent introduction of Myrtle Rust (caused by the fungus *Austropuccinia psidii)*. In Australia (Carnegie, Kathuria et al. 2016), myrtle rust can severely affect the plant's reproductive organs and reduce seed availability, to the extent that some species that have become classified as exceptional as viable seed can no longer be collected (Dalziell, Funnekotter et al. 2025). Tissue culture and cryobiotechnology research is essential to overcome the challenges needed to conserve this diverse family, where many of the tropical and rainforest Myrtaceae producing desiccation sensitive seeds require cryopreservation for long-term conservation (Hardstaff, Sommerville et al. 2022, Bao, O'Donohue et al. 2024). While cryopreservation has proven to be a vital ex-situ conservation tool for Australian species, with 145 species having been successfully cryopreserved (Funnekotter, Hardstaff et al. 2021), many of the newly identified exceptional species have yet to be tested and are a priority for future cryo-conservation research.

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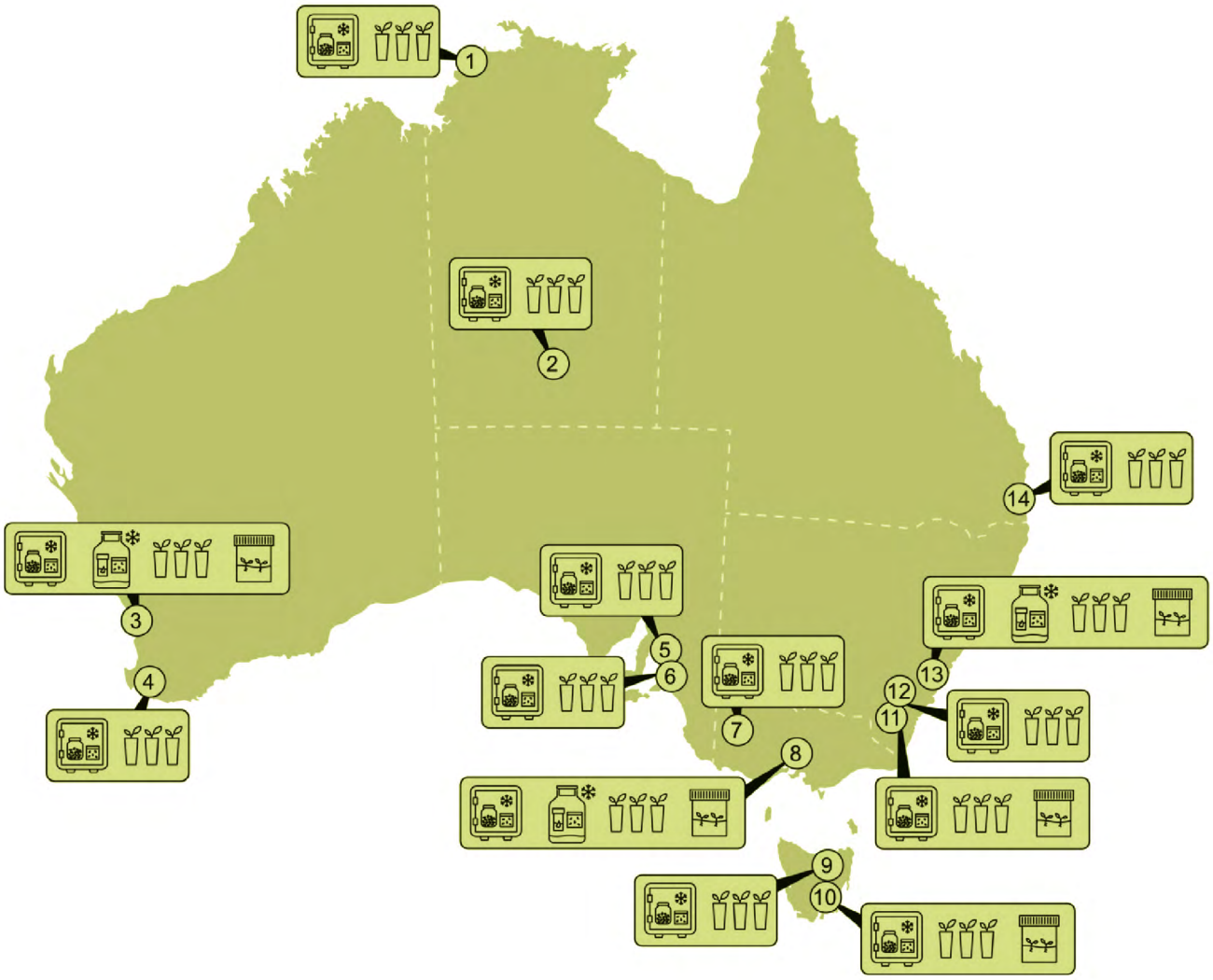
"Australia is home to more than 21,000 plant species, with 1,482 listed as threatened and in need of conservation."
(DCCEEW 2025)



Seedbanking

Cryostorage

Nursery Production

Micropropagation

Figure 1. *https://nph.onlinelibrary.wiley.com/doi/full/10.1002/ppp3.10421*

Thailand

Current Status and Scale of Cryobiotechnologies for Exceptional Plant Conservation

Supaporn Rodpradit

Queen Sirikit Botanic Garden,
The Botanical Garden Organization,
Thailand

Introduction

Thailand, a biodiversity-rich tropical country in Southeast Asia, is home to approximately 11,000 plant species. However, many species are at risk of extinction, with 221 species assessed as globally threatened on the IUCN Red List. Exceptional plant species, which cannot be conserved through conventional seed banking, pose a particular conservation challenge. Thailand has identified 531 exceptional species, including 14 classified as threatened (Pence et al., 2022b). Cryopreservation offers a promising long-term strategy for safeguarding these species.

Development and Research on Cryopreservation in Thailand

Research efforts in Thailand have primarily focused on orchids, as the country hosts 1,319 native orchid species and is a leading global exporter. Some orchids have been categorized under Exceptionality Factor 3 (EF3), indicating that their seeds are desiccation tolerant but cannot survive long-term storage at −20 °C (Francisqueti et al., 2024; Pence *et al*., 2022a). To date, successful cryopreservation has been reported for 31 orchid species across 18 genera, using various explants such as pollinia, seeds, protocorms, protocorm-like bodies, and somatic embryos. Techniques employed include desiccation, vitrification, encapsulation-dehydration, encapsulation-vitrification, droplet-vitrification, and the D- and V-cryo-plate methods (Thammasiri, 2020).

In addition, cryopreservation research has expanded to economically important crops, including oil palm (Khawniam & Te-chato, 2011, 2012), taro (Rukkid et al., 2021), and coconut (Thananchai et al., 2024). Notably, *Magnolia sirindhorniae*, a Critically Endangered species endemic to freshwater swamp forests, has been successfully cryopreserved (Kesa et al., 2006).

Major plant cryopreservation research is conducted by leading academic institutions such as Mahidol University, Prince of Songkla University, and Kasetsart University, as well as government agencies including the Department of Agriculture Gene Bank (DOA Gene Bank), the National Biobank of Thailand (NBT), which focus on economic crops, and the Queen Sirikit Botanic Garden (QSBG), which concentrates on orchids and other threatened species.

Current efforts at QSBG include exploring simplified cryopreservation methods for preserving orchid seeds and pollen. Seeds from 14 species across 7 genera and pollen from 21 species across 12 genera have

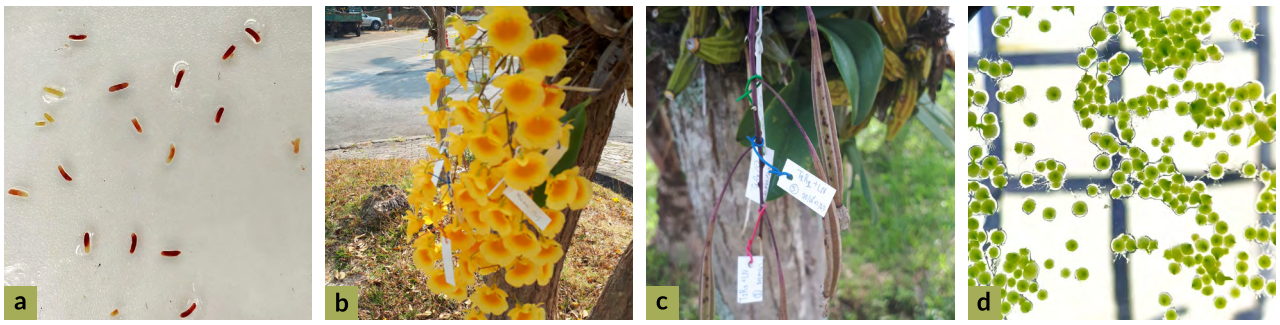


Figure 1. *Cryopreservation of Dendrobium lindleyi's pollinia. (A) Cryopreserved pollinia stained red, indicating viability. (B) Orchid flower pollinated with cryopreserved pollinia (Photo by: Wattana Tanming). (C) Capsule formation 6 months after pollination. (D) Seed germination after 1 month of culture.*

Figure 2. *Micropropagation of Magnolia thailandica, a dioecious, vulnerable species endemic to Thailand.*

(A) Male flower (Photo by: Charan Maknoi)

(B) Shoot elongation after 5 months of culture from a shoot tip with an axillary bud

been successfully preserved. Furthermore, QSBG has initiated the development of micropropagation protocols for *Magnolia* species as a preparatory step toward cryopreservation. Thailand harbors 27 *Magnolia* species, four of which are globally threatened. Many of these species produce limited quantities of viable seeds, suggesting classification under Exceptionality Factor 1 (EF1)—species that either do not produce seeds or produce seeds with extremely limited quantity or viability (Pence et al., 2022a).

Challenges and Future Directions

Despite these advancements, the application of cryopreservation in Thailand remains limited to laboratory-scale initiatives. Key challenges include insufficient policy support, inadequate infrastructure, and a shortage of trained specialists. Moreover, research has primarily focused on economically valuable species, whereas families facing critical conservation challenges such as Rutaceae, Fagaceae, Dipterocarpaceae, Arecaceae, and Fabaceae—have received limited attention.

To significantly advance the conservation of exceptional plant genetic resources in Thailand, it is imperative to strengthen both national and international collaborative networks for cryobanking. Such collaborations will support global plant conservation and contribute to a sustainable future.

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China

Plant Cryopreservation: Research, Applications, and Prospects

Lin Liang

The Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, China

China hosts a network of five germplasm conservation facilities, which preserve both crop and wild species. These include facilities for both conventional seed banking as well as cryopreservation, and they also support ongoing research for improving and applying cryobiotechnologies to a wide range of taxa.

The **Germplasm Bank of Wild Species** (GBOWS) of China in Kunming, Yunnan, is a national facility for the preservation of wild germplasm resources. The Seed Bank of GBOWs opened in 2008 and since then has preserved more than 10,000 species from across China. The GBOWS started cryobiotechnology research in 2009 and the GBOWS cryobank started functioning in 2019, and this is expanding in 2025. Research has included cryopreservation of shoot tips, embryogenic cell lines, short-lived seeds, protocorm-like bodies, and pollen, and work has focused on *Magnolia spp*, orchids, gesneriads, and other species. Stimulating bud production on leaf discs of several Gesneriaceae species provided shoot tip clusters that survived cryopreservation better than traditionally isolated shoot tips of those species. They also found that cryostorage extended the lifespan of short-lived seeds and pollen over other storage temperatures.

The **National Crop Gene Bank of China** in Beijing focuses on developing protocols for preserving crop species, and more than 1200 accessions of potato, banana, lily, taro, yam, carnation, Jerusalem artichoke, and other species have been cryopreserved, primarily as shoot tips. They have also developed slow growth protocols for potato, banana, lily, and other species.

The **National Gene Bank of Tropical Crops** in Hainan Province has focused on developing cryopreservation procedures for banking shoot tips and green globular bodies of tropical crop and endangered species, including *Cassava*, *Musa*, *Millettia speciosa*, *Neottopteris nidus*, *Morinda officinalis*, and *Aquilaria sinensis*.

The **National Germplasm Bank of Medicinal Plants** in Haikou Hainan Province preserves primarily tropical medicinal plants. They have developed protocols for cryopreserving 12,000 accessions of recalcitrant seeds of 170 species of medicinal plants. They established a technical framework for recalcitrant seed cryo-preservation and published national standards and monographs for cryopreservation of seeds of medicinal plants.

> "China hosts a network of five germplasm conservation facilities."

The **National Forestry and Grassland Germplasm Resources Facility** in Xiong'an, Hebei Province, is under construction and expected to open in 2028 with a potential capacity of 1.8 million accessions, with the focus on cryopreserving forest and grassland species.

One of the teams that will support this work will be Professor Liu Yan, from the **Beijing Forestry University.** Professor Liu's research group has focused on pollen cryopreservation, but has also worked with shoot tips, protocorm-like bodies, and seeds. She has established a pollen bank for over 100 ornamental species, including Paeonia.

To summarize, cryopreservation research has increased since 2010, covering multiple aspects of plant cryopreservation. More cryogenic facilities have been established, and the investment in research and preservation have also increased. These have formed the China Plant Cryopreservation Network for Conservation and Innovation, and they look forward to developing collaborations with international colleagues in the future.

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India
Exceptional Plant Conservation

Anju Mahendru-Singh

Division of Germplasm Conservation
ICAR–National Bureau of Plant Genetic Resources, India

India is listed among the top megadiverse countries and is home to over 47,000 plant species which includes 17,500 species of higher plants. The National Bureau of Plant Genetic Resources (NBPGR), New Delhi, an apex Institute of the Indian Council of Agricultural Research, is responsible for conserving the plant genetic resources that are important for food and agriculture including their wild and weedy relatives in the country. The National Plant Genetic Resources Network is spearheaded by NBPGR along with its 10 regional stations and the National Active Germplasm Sites across diverse agro-climatic zones of the country. Explorations organized by NBPGR along with these partners to different parts of the country bring in the diverse germplasm. National Genebank, the world's second-largest (Figure 1), is managed by the Division of Germplasm Conservation of NBPGR for conserving seeds of the orthodox and the recalcitrant seed species, as well as the vegetatively propagated species (https://nbpgr.org.in/nbpgr2023/genebank-status-2/). 0.47 million accessions of orthodox seeds belonging to more than 2000 species are conserved in -180°C in the seed genebank. Conservation of exceptional plant

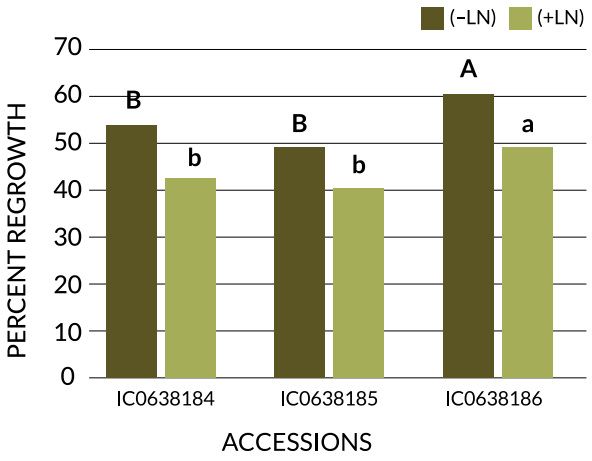
NATIONAL GENEBANK

Figure 1. *National Genebank of India at ICAR–NBPGR, New Delhi safeguarding plant genetic resources wealth of India*

> "India is listed among the top megadiverse countries and is home to over 47,000 plant species which includes 17,500 species of higher plants."

species is carried out in the *in vitro* (IVG) and cryo genebanks. The IVG conserves diverse crops through tissue culture in the *In Vitro* Active Genebank (IVAG) for short- to medium-term storage and through cryopreservation in the *In Vitro* Base Genebank (IVBG) for long-term storage. IVAG holds 2,056 accessions (>40,000 cultures) across six crop groups, including tropical fruit crops (449), temperate and minor tropical fruit crops (420), tuber crops (530), bulbous and ornamental crops (190), medicinal and

aromatic plants (232), and spices and industrial crops (235) belonging to 182 exceptional species falling under 69 genera. IVBG holds 347 accessions cryopreserved using shoot tips/ meristems or shoot bases. Cryopreservation protocols have been developed/refined using *in-vitro/ex-vitro* derived shoot tips/meristems. Initially, success was achieved in two genera, namely *Allium* and *Musa.* The droplet-vitrification method by optimizing the duration of treatment of sucrose and PVS2 was established. Subsequently, success was achieved with several



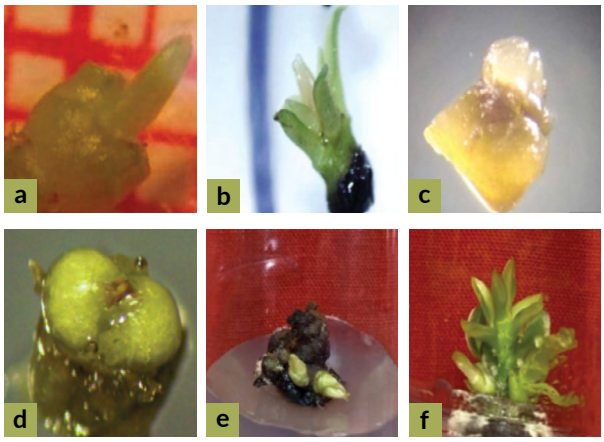
Regrowth percentage obtained by applying the standardized protocol to three different accessions of Garcinia indica.

- Germplasm conservation using droplet vitrification technique of cryopreservation in accession IC638183 standardized with 51.75% regeneration
- The technique was validated on three other G. indica accessions (IC0638184, IC0638185, IC0638186) using in vitro derived shoot-tips

Figure 2.1 *Refinement of cryopreservation protocol in Garcinia indica*

other species such as *Bacopa*, *Citrus*, *Dahlia*, *Dioscorea*, *Ensete*, *Gentiana*, *Garcinia*, *Gladiolus*, *Hylocereus*, *Humulus*, *Malus*, *Morus*, *Musa*, *Picrorhiza*, *Pyrus*, *Prunus*, *Rubus*, *Vaccinium*, *Simondsia*, *Valeriana* etc. (Figure 2).

In addition to the tissues, protocols for other explants such as embryos and embryonic axes, dormant buds, pollen as well as the non-orthodox



A. *Apical shoot-tip;* ***B.*** *Shoot induction and survival at 40 min. PVS2 treatment;* ***C.*** *Surviving ST after Droplet vitrification* ***D-F.*** *Shoot regeneration and proliferation after Droplet vitrification. (Bar represents A to D = 1mm & E-F = 1cm)*

Figure 2.2 *Refinement of cryopreservation protocol in Garcinia indica*

seeds have been developed in-house and are being deployed to conserve several of the exceptional species such as *Ardisia macrocarpa*, *Cornus capitata*, *Corylus ferox*, *Docynia indica*, *Dillenia indica*, *Etlingera fenzlii*, *Eriobotrya japonica*, *Maclura cochinechinensis*, *Catunaregam spinos*, *Musa spp.*, *Prunus napaulensis*, *Pyracantha cranulata*, *Pyrus pashia*, *Sorbus cuspidata*, *Zanthoxyllum armatum*, *Tamilnadia uliginosa*, *Vaccinimum glaucoalbus*, *Prunus*, etc. Desiccation and freezing sensitivity studies have been carried out and critical moisture content for successful cryoconservation deciphered for a number of other species. As a result, more than 13,101 accessions of 1000 species including orthodox and non-orthodox seeds and embryos (12,025), dormant buds (389) and pollen (686) have been cryoconserved in the cryogenebank at NBPGR.

Regular monitoring of the viability and regeneration of conserved accessions takes place at NBPGR. Various regenerated accessions have been established in the Field Genebanks (Figure 3) and supplied to the researchers across the country.

National and international trainings of 10-15 days duration at regular intervals are conducted by the Division of Germplasm Conservation at NBPGR. We look forward to collaborations in strengthening knowledge-sharing and protocol development for more plant species.

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ISSAPUR FARM **FIELD GENEBANK**

Plants of Pithecellobium dulce (manila tamarind) and of Tamarindus indicus (tamarind) raised from cryopreserved seeds successfully established at Issapur Farm of NBPGR

Cryo Genebank

25 plantlets (post cryostorage) Carissa carandas, Phoenix dactylifera, Pithecellobium dulce, Tamarindus indica

Initial establishment

Figure 3.1 *Examples of successful nursery and field establishment of plant genetic resources conserved in the National Genebank, India*

VIGNA VEXILLATA: **WILD COWPEA**

6–26 years of cryopreservation

S. No.	EC/1C/ Coll. No.	Date of Conservation
1	1C 277034	7.2.2001
2	1C 277055	29.1.2002
3	1C 139290	13.1.1994
4	LOCAL	25.7.1995
5	1C 235907	19.1.2000
6	1C 277035	29.1.2014
7	1C 331617	29.1.2002

100% Germination

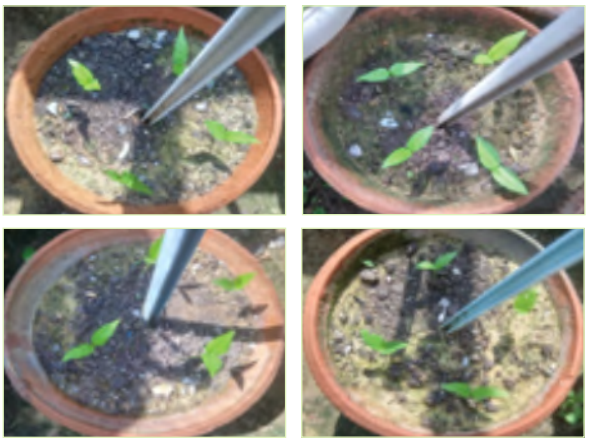

Figure 3.2 *Examples of successful nursery and field establishment of plant genetic resources conserved in the National Genebank, India*

South Africa

Exceptional Plant Cryobiotechnologies: Current Status and Future Directions

Shakira Shaik

University of KwaZulu-Natal, South Africa

The African continent comprises around 45,000 plant species in various biogeographical regions divided into many diversity hotspots. However, this diversity is unevenly distributed with southern Africa being disproportionally species rich. In particular, South Africa is home to approximately 30,000 species, and three biodiversity hotspots viz., the Cape Floral Region, the Succulent Karoo, and the Maputaland-Pondoland-Albany Hotspot. Many species are endemic, and threatened by climate change, habitat loss, and overexploitation. Despite the richness and threats, cryobiotechnological efforts to conserve exceptional species remain limited and fragmented across the country.

In a review of the state of exceptional plant cryopreservation in South Africa, the current institutional players are the South African National Biodiversity Institute (SANBI), the Agricultural Research Council, the South African Sugarcane Research Institute, universities, botanical gardens, nurseries, and the newly emerging Biodiversity Biobank South Africa. In the past, one of the key researchers driving cryopreservation research was Professor Patricia Berjak (University of KwaZulu-Natal), who pioneered work on recalcitrant seeds, and also authored the last cryoconservation update in 2011. Since then, exceptional species falling under various categories—few or no seeds for banking or clonally propagated (EF1), recalcitrant (EF2), intermediate or short-lived (EF3) and dormant (EF4) seeds—have been successfully cryopreserved. These include South African indigenous species, viz., *Ammocharis coranica*, *Ekebergia capensis*, *Haemanthus montanus*, *H. albiflos*, *Protorhus longifolia*, *Siphonochilus aethiopicus*, *Solenostemon rotundifolius*, *Trichilia dregeana*, and *T. emetica;* introduced species, viz., *Acer pseudoplatanus* and *Castanospermum australe;* and one West African species, viz., *Dioscorea rotundata*.

Despite these successes, substantial challenges persist. These include, gaps in species-specific cryobiological knowledge, lack of long-term cryobanking infrastructure, regulatory barriers, and limited investment. Moreover, many threatened South African species fall into the EF2 and EF3 categories making cryobiotechnology critical for their long-term conservation.

Looking ahead, there is a need for national coordination of cryopreservation efforts, increased funding, integration between *ex situ* and *in situ* strategies, and strengthened collaboration with local communities, SANBI, and global partners. A dedicated training pipeline in cryobiotechnology through universities is also urgently needed. For South Africa to utilize both its biodiversity and foundational research in leading the conservation of exceptional species through cryobiotechnology, rigorous, strategic and well-funded efforts are required moving forward.

> "For South Africa to utilize both its biodiversity and foundational research in leading the conservation of exceptional species through cryobiotechnology, **rigorous, strategic and well-funded efforts are required moving forward."**

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Europe
The Kew Cryobank

Louise Colville

Royal Botanic Gardens, Kew, United Kingdom

With 45% of the world's plant species under threat of extinction, *ex situ* conservation provides an insurance policy against biodiversity loss when climate change, habitat destruction and geopolitical instability threaten *in situ* conservation efforts (Antonelli et al., 2023). Kew's Millennium Seed Bank (MSB) conserves wild species from around the world (currently holds c. 40,000 species) and holds comprehensive collections of wild species in the UK.

Whilst seed banking is suitable for many plant species, there are estimated to be c. 25-60% globally that cannot be conserved long-term in conventional seed banks such as the MSB (Pence et al., 2022). These have been termed "exceptional" species and includes species for which seeds cannot be collected due to inaccessibility or reproductive failure; recalcitrant seeds that do not survive drying; short-lived orthodox (desiccation tolerant) seeds; and seeds that cannot be easily recovered from storage due to complex dormancy or propagation requirements (Pence *et al.*, 2022). In addition to this, there are plants like ferns and mosses that do not produce seeds. These exceptional species require alternative methods such as cryopreservation to ensure their long-term security and potential for future use (Walters and Pence, 2021).

The Kew Cryobank will be established as a state-of-the-art research, conservation, and training facility for cryopreserving plant species that cannot be conserved in conventional seed banks. The focus in the first phase will be UK native plants and algae, including short-lived seeds (e.g., orchids), desiccation-sensitive seeds (e.g., oak, seagrass), non-seed-bearing plants (mosses, liverworts, ferns, horsetails, quillworts, pillworts, clubmosses), and algae (stoneworts and seaweeds). There are practical reasons for the focus on UK species, not least the challenges of shipping desiccation sensitive seeds and other material from overseas without loss of viability. However, there are also conservation reasons, with the potential to fill the gaps in MSB collections and secure the genetic diversity of UK native species, including those which are rare and threatened, and are currently not conserved in *ex situ* collections. Recent analysis has found that more than half of the 1,692 species of

Training course in cryopreservation at Kew in 2023. Photo by Louise Colville

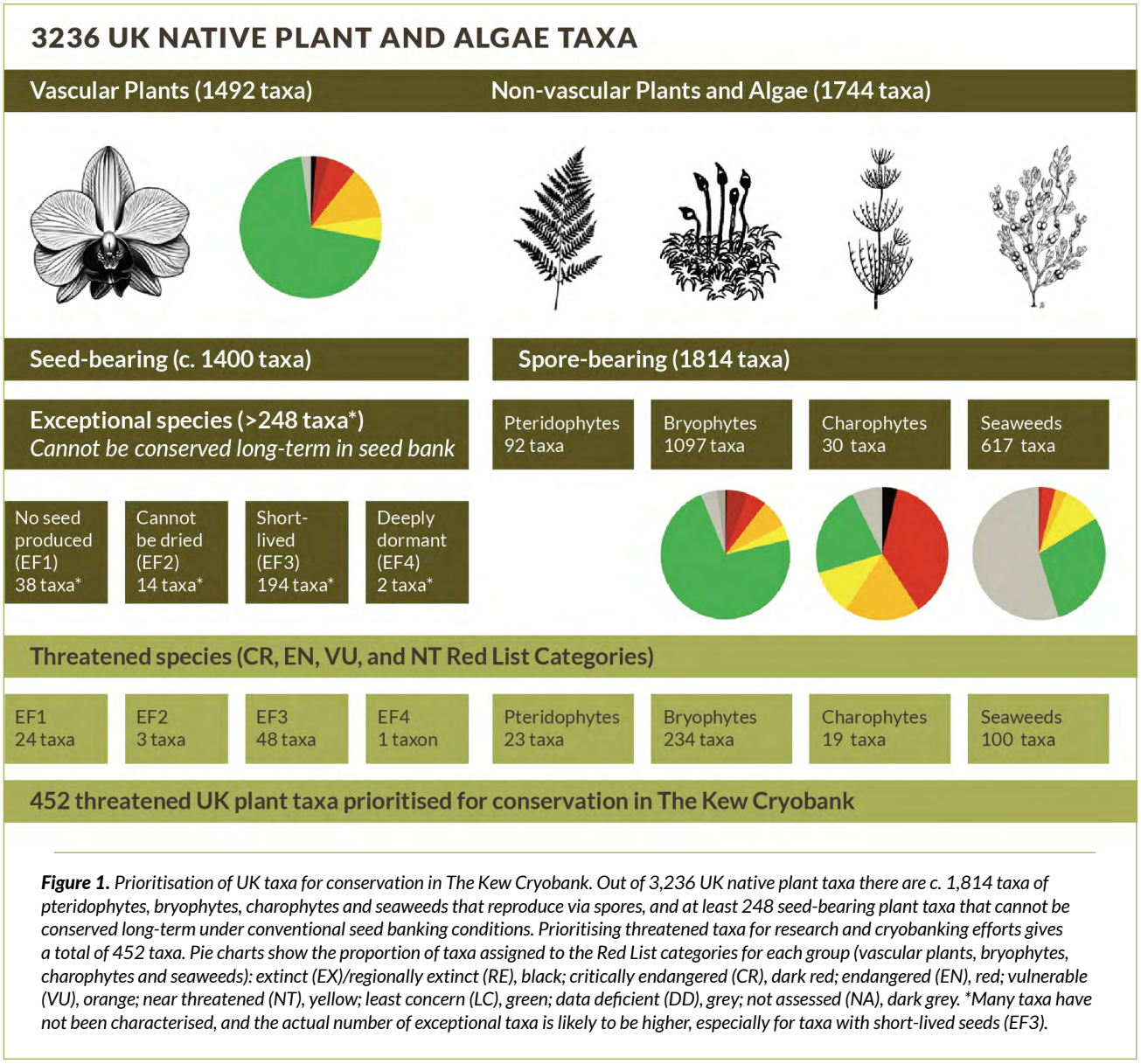

Figure 1. *Prioritisation of UK taxa for conservation in The Kew Cryobank. Out of 3,236 UK native plant taxa there are c. 1,814 taxa of pteridophytes, bryophytes, charophytes and seaweeds that reproduce via spores, and at least 248 seed-bearing plant taxa that cannot be conserved long-term under conventional seed banking conditions. Prioritising threatened taxa for research and cryobanking efforts gives a total of 452 taxa. Pie charts show the proportion of taxa assigned to the Red List categories for each group (vascular plants, bryophytes, charophytes and seaweeds): extinct (EX)/regionally extinct (RE), black; critically endangered (CR), dark red; endangered (EN), red; vulnerable (VU), orange; near threatened (NT), yellow; least concern (LC), green; data deficient (DD), grey; not assessed (NA), dark grey. *Many taxa have not been characterised, and the actual number of exceptional taxa is likely to be higher, especially for taxa with short-lived seeds (EF3).*

British native flowering plants, ferns and charophytes are in decline and there has been a significant increase in the distribution of modern introductions (neophytes) since 1950s (Walker et al., 2023). Some neophytes have become invasive, disrupting ecosystem function and outcompeting native species.

Beyond conserving genetic diversity, cryopreserved collections will provide a resource for research and propagation to support restoration activities. Cryobanking will be underpinned by cutting edge research in low temperature science, seed biology, and biodiversity conservation. Knowledge sharing and training will be a key component and will achieve a wide reach through delivery of international training courses, research placements and supervision of MSc and PhD students. This will help to strengthen cryobanking capacity worldwide to ensure that all plant species can be protected from the threat of extinction and preserved as a resource to realise their potential for future use in supporting *in situ* conservation and restoration.

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Europe
Cryobanking Agricultural Species

Bart Panis

Alliance of Bioversity and CIAT, World Banana Gene Bank, University of Leuven, Belgium

In this overview of cryopreservation activities for crops in Europe, three initiatives will be described: CRYMCEPT, CRYOPLANET and the Working Group on Cryopreservation of the European Cooperative Programme on Plant Genetic Resources (ECPGR).

The first, CRYMCEPT, was a research project conducted from 2002–2005, to establish new improved cryopreservation methods for conserving European germplasm collections. It involved partners from seven labs from across Europe. Specific objectives included the development of sensitive, reliable, and user-friendly techniques to measure responses to cryopreservation protocols and determine the physico-biochemical changes associated with tolerance towards cryopreservation. This should lead to the development of new protocols that could then be applied to the conservation of European germplasm collections.

The physico-biochemical parameters investigated included cytoskeleton proteins, membrane components, water thermal behaviour, protein, sugars, polyamines, and oxidative stress. These were studied in relation to treatments used to prepare tissues for cryopreservation and the stresses of cryoprotectant and liquid nitrogen exposure. The seven partner labs applied their analytical expertise in these seven areas using different crop species and came together at the end of the project to share their results in two workshops. Improved methods were applied to several crops, including banana and coffee.

Next, there was CRYOPLANET, which was a networking project involving 19 European countries. Some of the objectives included: to evaluate the current use of cryopreservation methods in Europe, then compare the efficiency of existing protocols, to improve knowledge of cryoprotection using the research from the CRYMCEPT project, to develop new cryopreservation protocols, to assure genetic stability of tissues through cryopreservation, to apply these methods to European germplasm collections, and to examine the environmental, social, and economic impact of cryopreservation. Two working groups were formed to deal with on the one hand the fundamental and on the other applied aspects of this project. There were many meetings of this group, including one that helped facilitate the first ISHS International Symposium on Cryopreservation, held in Leuven in 2009.

Finally, there is the European Cooperative Programme for Plant Genetic Resources. This is also a network, but with working groups focused around specific crop species. More recently, in 2022, another working group was added focusing on a technique, namely cryopreservation, with its first meeting held in 2023 in Prague. Since then, there have been training and workshop events held for dormant bud and garlic cryopreservation, and in 2025, a regional cryobanking project for the Western Balkans and the Caucasus was initiated.

The objectives of the European Cooperative Programme are similar to those of CRYOPLANET but further emphasize maintaining cryopreservation research in Europe at a critical mass to ensure further scientific advancements, applying cryopreservation to crop wild relatives, increasing collaboration, and creating cryopreservation backup facilities and data management systems.

Information on all of these programs can be found online, and some of the key cryopreserved crop collections in Europe involved in these projects are banana in Belgium, elm in France, garlic in the Czech Republic and Germany, and strawberry and potato, also in Germany. This work is the result of much collaborative work and many funding partners across Europe.

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Brazil and South America
Plant Cryopreservation

Neusa Steiner

Hawaiian Rare Plant Program, Harold L. Lyon Arboretum, University of Hawai'i at Mānoa, United States

Botany Department, Federal University of Santa Catarina, Brazil

South America territory is largely covered by tropical and subtropical biomes, which hold one of the highest plant biodiversities in the world (Wilson et al., 2021). This forest has an imperative role in the global carbon and water cycles (Pan et al., 2024).

Ex situ conservation programs of this plant's genetic diversity is a high priority since there is an alarming and progressive reduction of this ecosystem (Lima et al., 2020). Only 10% of the world's plants produce desiccation sensitive (DS) seeds, but in the tropics this number can be around 50% (Wise & Dicke, 2017). Plants that produce DS seeds, limited viable seeds or short life span when frozen at −20°C are called exceptional species (Walters & Pence, 2020). Conventional gene banks are not a suitable option for these species and specific approach are required to keep the long-term viability.

Cryobiotechnologies have been successfully used for the preservation of exceptional crops species from South America such as coffee, cocoa, cassava and potato (Nagel et al. 2024), but the majority of wild species are not even studied and many of them are threatened (Wilson et al., 2021). Myrtaceae, Arecaceae, Lauraceae and Fabaceae are typical tropical plant families with a high prediction of exceptional species (Pence & Bruns, 2022). Wild species from these families such as *Butia* spp, Bactris spp, *Euterpe* sp (Goeten et al., 2022; Steiner et al., 2026), *Catleya* sp, *Arachys* spp (Tacán et al., 2024) and *Passiflora* spp (Ferreira et al., 2024) have been successfully cryopreserved using seed tissues. Other species such as *Araucaria angustifolia* seeds are DS and a somatic embryogenesis protocol was required in order to cryopreserve embryogenic cultures (Demarchi et al., 2014; Fraga et a., 2016).

Universities National and International Agricultures Research Center in South America plays an important role in genebank crops and their wild relatives to keep food security. International Potato Center (CIP), in Peru, operates the world's largest potato cryobank. In Brazil, overall, more than 85 species from 23 families have been cryopreserved (Pacheco et al., 2025). In most exceptional species, the cryobiotechnologies approach relies on previous physiological studies in order to identify the water threshold as well as how morphological and biochemical composition could affect plant tissue survival in vivo and in vitro. To safeguard the tropical plant genetic diversity, more basic research studies in exceptional plant species as well as new technological tools and cryoprotectants, must be explored.

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Figure 1. *Native plants from Brazil: Butia catharinensis fruits (a), seed (b), and embryo (c); Araucaria angustifolia seeds (d), zygotic embryo (e), somatic embryos (f)*

Mexico

Agave as a Global Model for Conservation, Resilient Crops, and Climate Change Adaptation

Lourdes Delgado-Aceves

Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ), Mexico

Biodiversity erosion undermines economic stability, exacerbates poverty, and intensifies inequality, triggering a cascade of interconnected challenges that demand urgent global action (Sosa and Ivanova, 2025). In this context, Mexico plays a crucial role: it is recognized as one of the world's megadiverse countries, hosting approximately 10–12% of global species diversity (SEMARNAT, 2012). This richness includes a vast number of endemic plant species with significant ecological, cultural, medicinal, and economic value. However, much of this biodiversity is under serious threat due to anthropogenic pressures such as deforestation, climate change, overexploitation, and habitat loss, which have already contributed to an estimated 50% reduction in global land cover (Vazquez-Reyes et al., 2019).

> **"The future of *Agave* research** lies in expanding its genetic conservation, promoting sustainable cultivation practices, and diversifying its commercial applications."

The genus *Agave* represents a paradigmatic case due to its ecological, economic, and adaptive value. Native to arid and semi-arid regions, agaves have evolved unique physiological adaptations such as Crassulacean Acid Metabolism (CAM) that make them resilient to extreme environmental conditions (Nabhan et al., 2022; Ayil-Gutiérrez et al., 2025). By examining *Agave's* role in conservation, its resilience as a crop, and its contributions to climate adaptation, this study highlights the potential of *Agave* in addressing global environmental challenges and increasing agricultural innovation (Stewart, 2015; Alducin-Martínez et al., 2023).

This resilience has garnered global interest as an alternative for sustainable development across diverse contexts. In Mexico and Africa, agave is utilized for fiber production and soil erosion control. In Australia and Brazil, it contributes to an emerging bioeconomy in drought-prone regions. In the United States, the cultivation of agave for sweetener production and the adoption of sustainable agricultural practices are on the rise, with farmers in California and Arizona increasingly turning to agave as a water-efficient alternative to traditional, high-water-demand crops (Stewart, 2015; Davis et al., 2021). These diverse applications highlight agave's global significance and versatility.

The genus *Agave* faces significant challenges for its conservation and sustainable production due to its biological traits, including complex morphology, long life cycles, and its monocarpic nature, that is, flowering only once before dying (Bautista-Montes et al., 2022). *In vitro* culture has become a key tool for the rational management of plant species, enabling the development of strategies for large-scale propagation, as well as the long-term conservation of seeds, apical meristems, and somatic embryos (Figure 1). In recent years our lab has made significant advances in these areas (Delgado-Aceves et al., 2022; 2024a, b). We have shown very high survival of somatic embryos of *A. tequiliana* (83%) using the V-cryoplate method, as well as of shoot tips from in vitro cultures, using droplet vitrification for six rare or threatened *Agave* species: *A. peacokii* (97%), *A. karwinskii* (96%), *A. victoriae-reginae* (90%), *A. rzedowskiana* (86%), *A. guiengola* (75%), and *A. lurida* (75%) (Figure 2). This suggests that cryo-banking is a viable and important ex situ option for conserving *Agave* germplasm into the future.

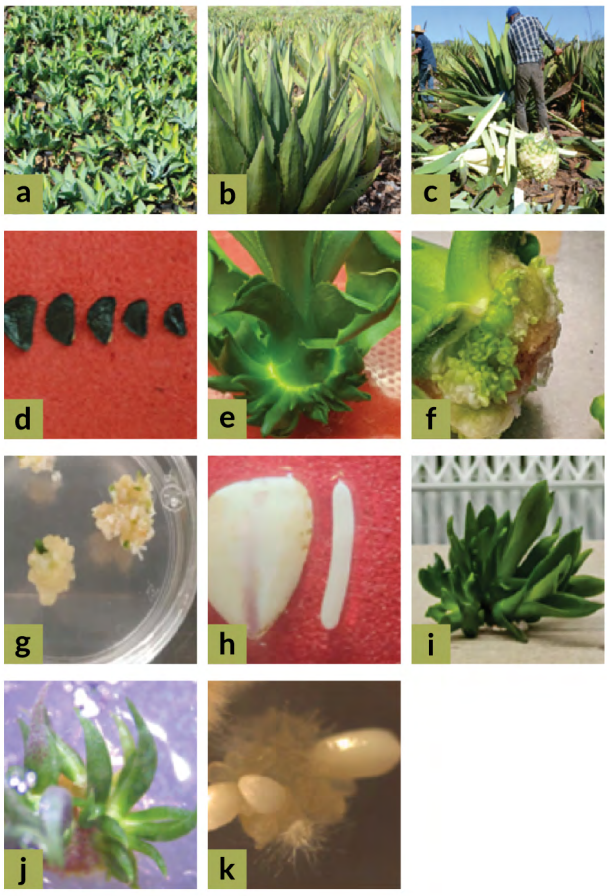

Figure 1. *Utilization and Biotechnological Development of Agave. a) Seedling cultivation in seedbeds. b) Semi-intensive cultivation c) Harvesting (jima) of mature plants d) Viable seeds e) Proliferation through axillary buds f) Shoot production via direct organogenesis g) Embryogenic callus with development of somatic embryos h) Endosperm and zygotic embryo i) Shoots developed via axillary bud proliferation j) Shoots developed via indirect organogenesis k) Somatic embryos at coleoptilar stage.*

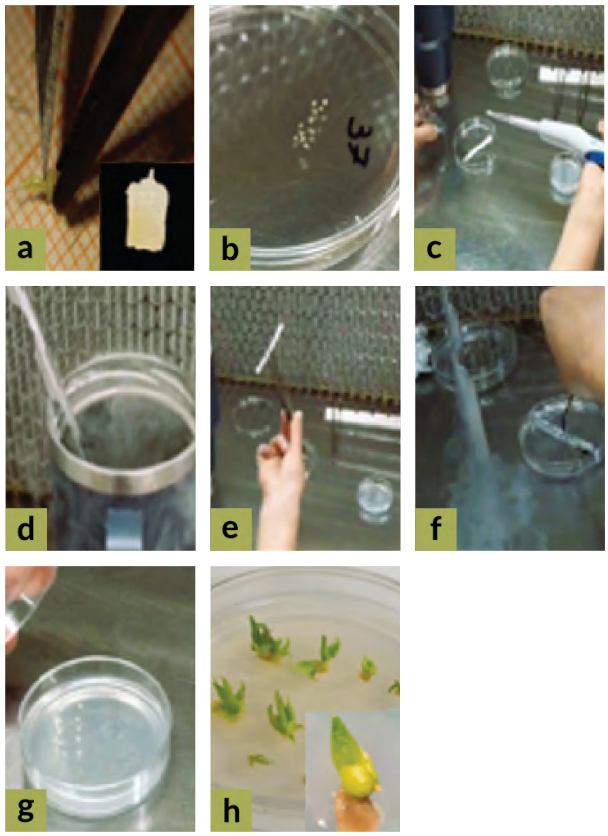

Figure 2. *Cryopreservation of Agave Apical Meristems. a) Dissection of apical meristem (upper right—close-up of apical meristem) b) Exposure to loading solution (LS) c) Application of vitrification solution (PVS2) in droplets onto aluminum foil containing apical meristems d) Container with liquid nitrogen e) Immersion of the aluminum foil in liquid nitrogen f) Recovery of apical meristems using recovery or warming solution (RS) g) Cultivation of apical meristems on semi-solid medium h) Post-cryopreservation apical meristems after 30 days of incubation (upper right—close-up of recovered apical meristem). Taken from Delgado-Aceves et al., 2024b.*

The future of *Agave* research lies in expanding its genetic conservation, promoting sustainable cultivation practices, and diversifying its commercial applications. Key areas of development include:

- Developing high-yield, climate-resilient varieties
- Improving sustainable harvesting techniques
- Investigating new markets for agave-based products
- Assessing *Agave's* potential role in ecosystem services
- Fostering global awareness and policy frameworks that recognize *Agave* as a climate-smart crop
- Strengthening both ex-situ and in-situ conservation programs to protect endangered *Agave* species and preserve genetic diversity long-term

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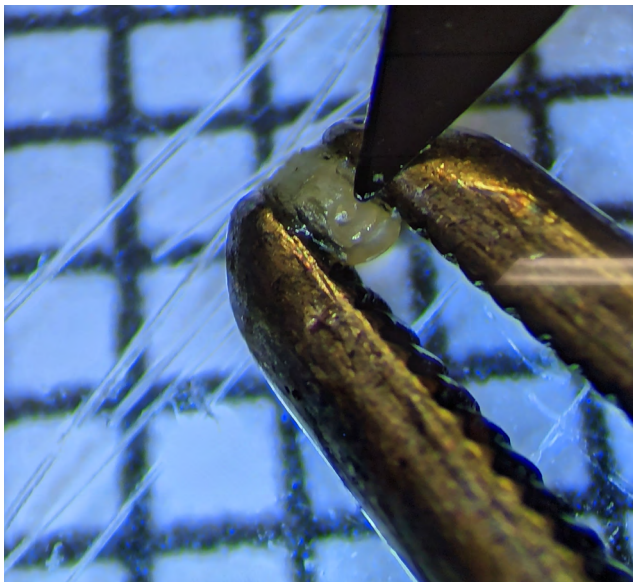
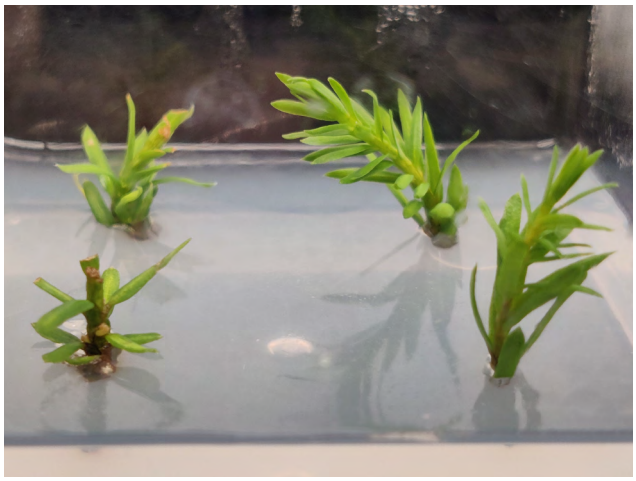
United States

In Vitro and Cryopreservation Strategies for Imperiled Plants of the Southeastern United States





Emily E.D. Coffey

Atlanta Botanical Garden,
United States

The Atlanta Botanical Garden (ABG) integrates cryopreservation into a multi-tiered ex situ conservation strategy to protect plant species at greatest risk of extinction in the Southeastern U.S. and Puerto Rico. This work supports national and global biodiversity frameworks, including the Global Strategy for Plant Conservation (GSPC) 2020–2030 and the Center for Plant Conservation's priorities.

Our in vitro program focuses on species with non-orthodox seeds and narrow geographic distributions, particularly exceptional *Magnolias*, *Quercus*, *Rhododendron*, *Torreya*, and native orchids. These taxa present challenges for conventional seed banking, necessitating alternative strategies like somatic embryogenesis, shoot tip culture, and long-term cryostorage in liquid nitrogen. We are actively developing protocols for these species, including the use of asymbiotic and symbiotic orchid micropropagation and cryopreservation of mycorrhizal associates.

Cryopreservation at ABG is not treated as an isolated action but is embedded within a broader conservation pipeline that includes seed banking, living collections, nursery-based propagation, and population augmentation. The decision to use cryo is made based on a species' reproductive biology, conservation status, and recovery trajectory. This integrated approach enhances resilience and supports restoration readiness.

Ongoing challenges include limited viability data for Caribbean endemics, the need for streamlined protocols for pre-freeze screening and post-thaw regrowth, and barriers related to permitting and access to material. To address these, ABG is developing a five-year roadmap to scale training, formalize and publish cryo protocols, and explore regional shared cryo infrastructure.

This work is supported by a strong cross-disciplinary team and robust partnerships through networks like the Global Conservation Consortium. We see cryopreservation as a cornerstone of the next generation of plant conservation efforts, especially as we face increased extinction risks from climate change and habitat degradation. Through innovation and collaboration, we aim to ensure that the most vulnerable plant species are not lost to time.

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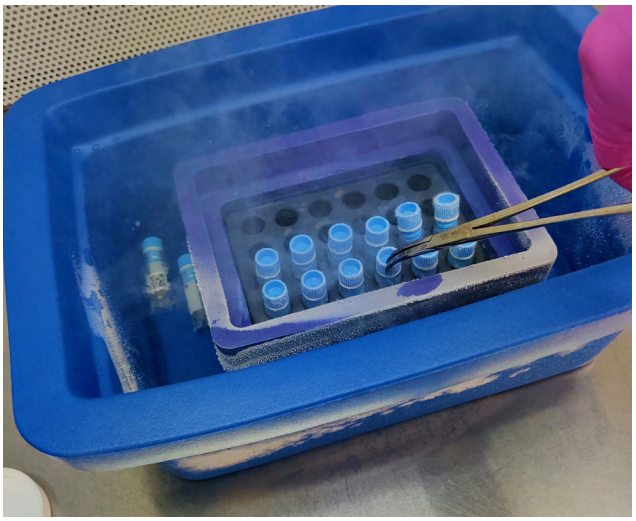
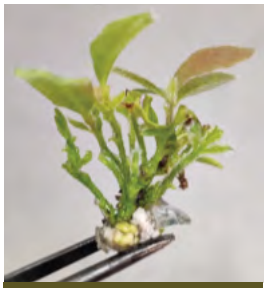


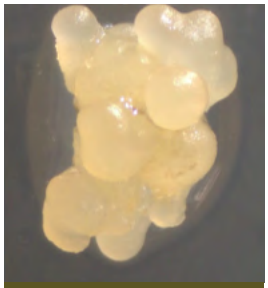
IN VITRO: **CRYOPRESERVATION**



Quercus boyntonii

Quercus georgiana

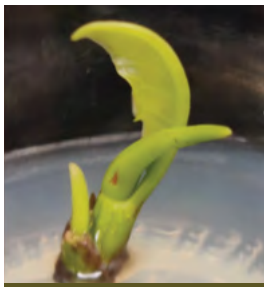
Quercus oglethorpensis



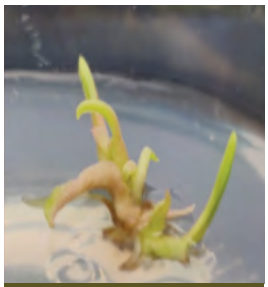
Somatic embryogenesis

Magnolia ashei

Magnolia pyramidata



Magnolia portoricensis



Magnolia splendens

United States
In-Vitro Storage and Cryopreservation of Exceptional Plants

Devon Gordon

Hawaiian Rare Plant Program,
United States

The Hawaiian archipelago is home to approximately 1400 species of plants, 425 of which are listed as endangered by the United States Fish and Wildlife Service (USFWS). Over half of these endangered plant species have less than 50 individuals remaining in the wild, making them critically imperiled with extinction. Many of these species are considered exceptional, mostly because they are either recalcitrant, short lived in seed storage, or seeds are not accessible. A network of plant conservation partners around Hawaii work towards the goal of conserving these critically imperiled species from extinction. The Hawaiian Rare Plant Program (HRPP), a research facility of the University of Hawai'i at Mānoa and located at the Lyon Arboretum, maintains a germplasm repository to safeguard critically endangered plant species. The mission of the HRPP is to prevent further extinctions by preserving these species through its Seed Conservation Lab and Micropropagation Lab. In the Micropropagation lab, exceptional plant species are held in-vitro as the primary method of long-term storage since its inception in 1992. The in-vitro collection contains 50,000 individual plants, made up of 170 USFWS listed species, 22 of which are extinct in the wild. Since 2019, the Micropropagation Lab has begun developing protocols for cryopreservation of its exceptional plant species held in-vitro, to extend their longevity and increase labor efficiency over the long-term.

The cryopreservation program has targeted the most critically imperiled species held in-vitro, such as the extinct in the wild *Phyllostegia kaalensis*, for protocol development and secure storage in liquid nitrogen. More exceptional species are continuing to be targeted by the program for cryobanking, allowing for the secure long-term storage of exceptional Hawaiian plant species.

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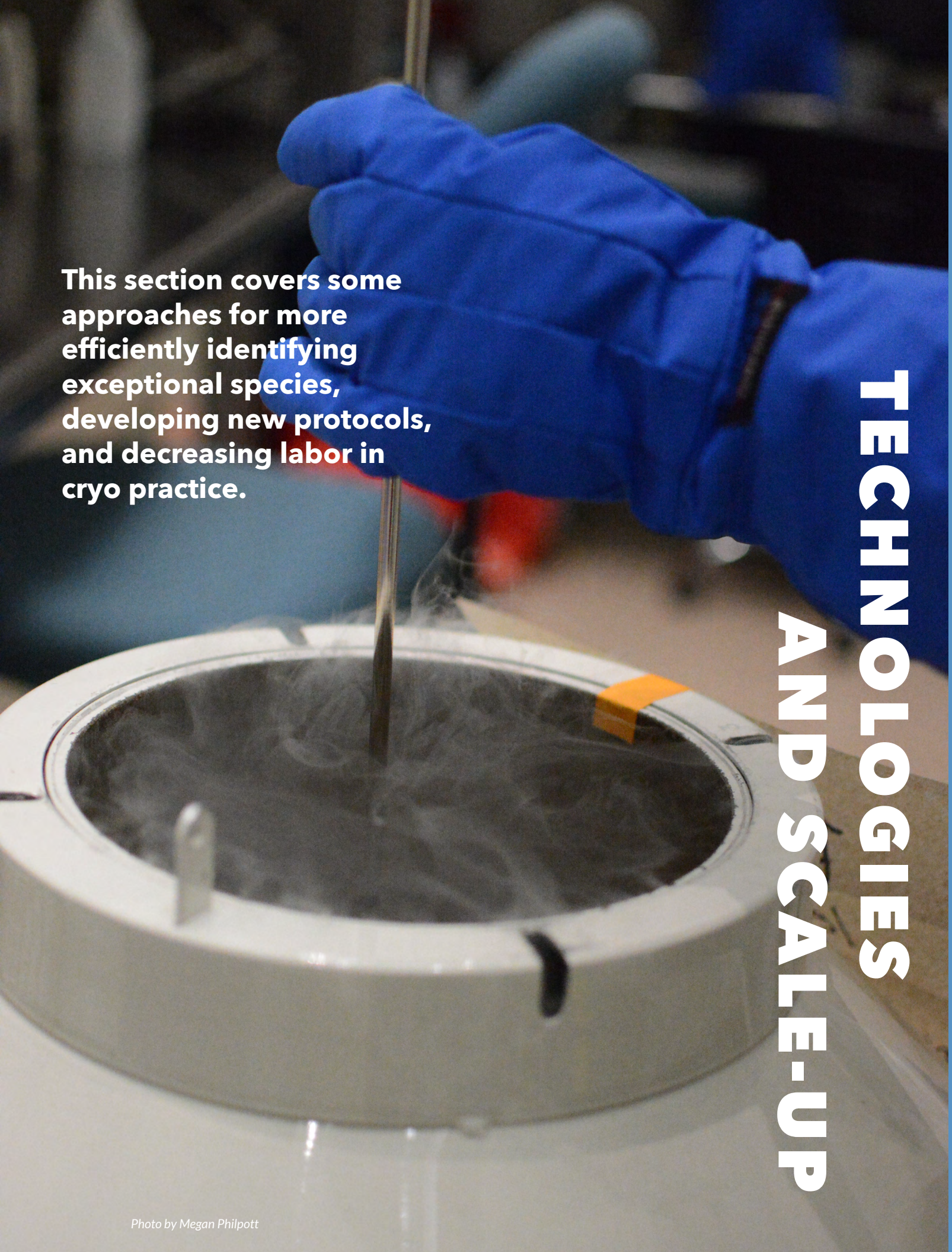
HRPP Micropropagation Lab



TECHNOLOGIES AND SCALE-UP

This section covers some approaches for more efficiently identifying exceptional species, developing new protocols, and decreasing labor in cryo practice.

Photo by Megan Philpott

Identifying
Exceptional Species

Karen Sommerville

Botanic Gardens of Sydney, Australia

The decline in rainforest extent and diversity has led to an increased need for ex situ conservation to provide insurance against extinction and support habitat restoration. Seed banking provides one option for conservation but information regarding how rainforest seeds will respond to drying and cold storage, and how long they're likely to survive in storage, is often lacking.

The Rainforest Seed Conservation Project commenced in 2013 with the aim of assessing germinability, storage behaviour and longevity for seeds of Australian rainforest plants. Data for 162 species (representing 63 families) were published in 2021 and indicated 64% were tolerant of drying to equilibration with 15% relative humidity, and another 10% were at least partially tolerant. Easily measured characteristics such as seed moisture content, dry weight and seed coat ratio were found to be useful, at either end of their range of values, for predicting the response to drying. This led to the development of a 7-step key, combining our own results with those of earlier studies, to provide a means of assessing desiccation tolerance when seeds are scarce or hard to germinate (Sommerville et al. 2021). As modelling has shown that genus is a good predictor of desiccation response (Wyse and Dickie, 2018), the first step in the key is to check for information on storage behaviour for other species in the same genus. Data for many genera may be found in the Seed Information Database (https://ser-sid.org). If no data are available, or the available data are inconclusive, the next steps involve assessing whether seeds have a soft or hard seed coat and, if hard, whether the seed coat is impermeable to water, which is a good indicator of desiccation tolerance (Tweddle et al. 2003). If the seed coat is permeable, one weighs a subsample of seeds before and after drying in an oven to determine percentage moisture content and dry weight. Seeds with a moisture content ≤ 20%, or a dry weight ≤ 20mg, are usually desiccation tolerant while those with a moisture content > 50% and a dry weight > 20mg are usually desiccation sensitive (Sommerville et al. 2021). If moisture content and dry weight fall outside that range, one goes on to the next step in the key and so on until a likely response to desiccation is determined.

The key described above is useful for assessing the response to desiccation but not the response to storage. As around a quarter of the species identified as desiccation tolerant in this study were later found to be very short-lived in storage at -20°C (Sommerville et al. 2021, 2023), we used thermal analysis to investigate the contribution of lipid transitions to poor longevity. Short-lived species were found to have distinct lipid crystallization and melting transitions around -20°C. Storage of seeds at temperatures above or well below the observed transitions have led to improved longevity for species in the Myrtaceae, Melastomataceae, Pittosporaceae and Proteaceae (Sommerville et al. 2025). These research tools are improving our capacity to conserve Australian rainforest species and may also be more broadly applicable.

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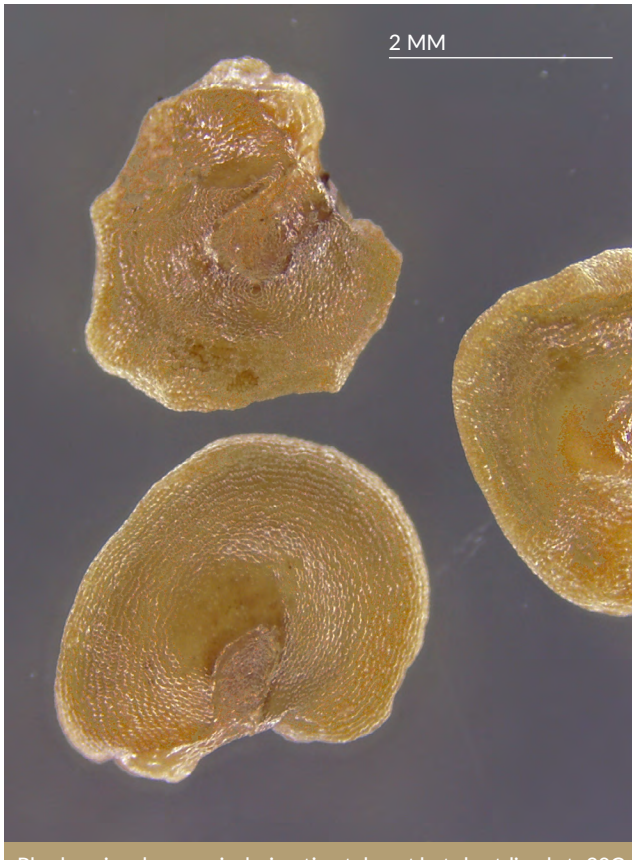
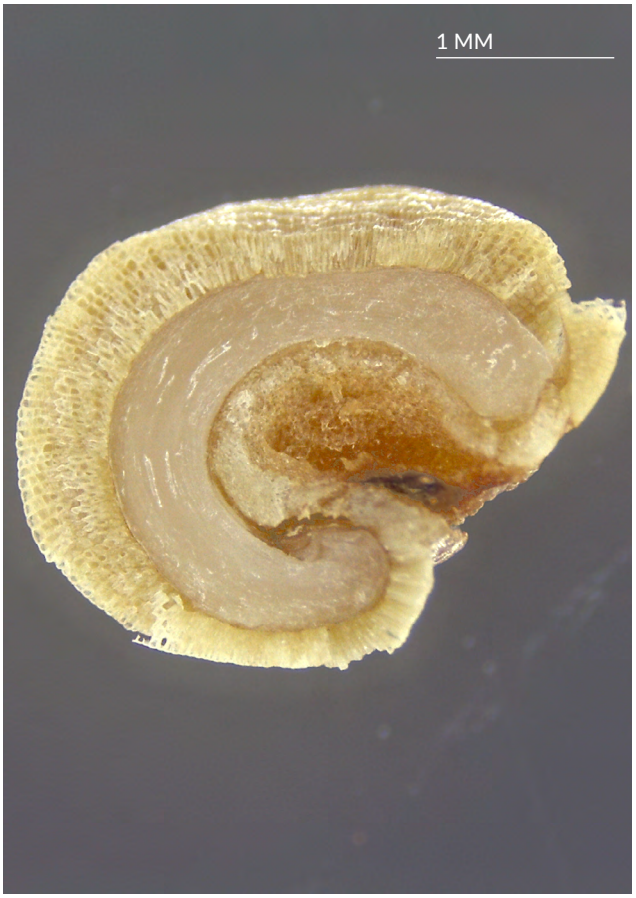
Syzygium hodgkinsoniae is recalcitrant



Rhodamnia rubescens is desiccation tolerant but short-lived at -20C



Triaging Exceptional Species
at the Millennium Seed Bank

Sharon Balding, Rachel Davies, Sarah Gattiker, & Nicola Mills

Millenium Seed Bank, Royal Botanic Gardens, Kew, United Kingdom

The Millennium Seed Bank (MSB) represents the greatest concentration of living seed-plant biodiversity on earth. This wild seed bank is a global resource for conservation, research and the sustainable use of plants. With 100,000 collections, it currently holds over 40,000 species and nearly 2.5 billion seeds.

Although the seeds of many species can be stored and conserved under our normal seed bank conditions of -20°C after drying at 15% relative humidity (RH), it is not suitable for a significant proportion of exceptional species, such as desiccation sensitive (recalcitrant) or freeze sensitive seeds, and may not provide optimal conditions for species identified as short-lived.

External view of the Millennium Seed Bank Jim Holden © Board of Trustees, RBG Kew

Millennium Seed Bank cold room storage Wolfgang Stuppy © Board of Trustees, RBG Kew

A germination test on an agar plate

Seed storage in the Millennium Seed Bank © Board of Trustees, RBG Kew

The difficulties with banking some exceptional species influences the targeting and collection of species which are destined to be stored at the MSB, with partners aiming to avoid collecting recalcitrant seeds by compiling target lists of orthodox (desiccation tolerant) species. Pre-import checking procedures also include checks against known and suspected short-lived species, so we are prepared for the processing requirements when the seeds arrive. Very short-lived species (such as Orchidaceae) need to be sent for processing very quickly–within two weeks of collecting.

Seed collections are triaged on arrival at the MSB with the short-lived species undergoing speedier processing times and then routinely duplicated to cryo-storage at a higher RH, where seed life spans are predicted to be considerably longer. We use the literature, research and our own seed bank data to determine these short-lived taxa. By storing seed collections in two different conditions, we can study the impacts of both and safeguard the collections.

We describe the process we undergo when working with short-lived and very-short-lived species arriving at the MSB and provide examples of some of the relative humidity levels and the percentage quantities split between cryo-storage and normal banking that we have adopted for certain genera.

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Low(er) Hanging Fruit for **Cryobanking In Vitro Tissues**

Valerie Pence & Megan Philpott

Center for Conservation & Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, United States

Cryobanking is a necessary tool for the long-term ex situ conservation of exceptional species, but the time and resources required, in comparison with seed banking, are considerable. Choosing the most efficient prioritization strategies will be critical for ensuring the long-term conservation of threatened exceptional species. Projects to conserve exceptional species using short-lived seeds and pollen are underway, and these have often been called "low hanging fruit," because they can be handled similarly to seeds for conventional seed banking. Species that must be conserved with methods that require more resources, such as those requiring in vitro steps, are considered more challenging, but within this group there are those that may be less challenging than others.

Exceptional species related to species with known cryopreservation and in vitro protocols could be prioritized. We know that some species adapt to conventional methods for in vitro growth more readily than others. Examples from our laboratory include *Hedeoma todsenii* (Lamiaceae) and *Spirea virginiana* (Rosaceae), which have been initiated into culture, cryopreserved, regrown, and acclimatized using relatively standard protocols. *Asimina tetramera* (Annonaceae), on the other hand, required additional research for successful rooting, while species of *Quercus* (Fagaceae), have been more challenging at the outset, being difficult to initiate into culture. Using known protocols could direct work for related species that are threatened and exceptional.

In a recent study, it was shown that of the 366 genera currently known to contain exceptional species, 186 were not represented in the literature; on the other hand, 180 *were*. Of the 20 species that were represented most in the literature, all were economically important woody species with either recalcitrant or short-lived seeds, and these traits are often shared by congeners and also often indicate that in vitro methods will be needed for conservation. These 20 have 343 known threatened congeners and likely many share the same seed storage characteristics and could be prioritized.

Research directed at increasing the speed and efficiency of cryobanking is also needed. Harnessing the regenerative power found in the leaves of many species is one area that could be fruitful. Regenerating buds from leaves can be quicker than establishing and building stocks of shoot cultures, and preparing such buds for cryopreservation has been shown to require only 10-20% of the time required for conventional shoot tip isolation. This approach has been demonstrated with several crop species, as well as species within the family Gesneriaceae, which shows a high capacity for leaf regeneration over many species. Prioritizing threatened exceptional species, such as those that can be cryopreserved more efficiently, can also increase efficiency.

> "Choosing the most efficient prioritization strategies will be critical for ***ensuring the long-term conservation of threatened exceptional species.***"

Taken together these and similar approaches could help advance the conservation of the next level of exceptional species. These might not be considered "low hanging fruit" but perhaps they represent "lower hanging fruit" than the most problematic species.

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Systematic Approach to **Developing Cryopreservation Protocols** for New Plant Materials

Elena Popova & Haeng Hoon Kim

K.A. Timiryazev Institute of Plant Physiology of Russian Academy of Sciences, Russia

Department of Agricultural Life Science, Sunchon National University, Korea

Despite significant progress in cryopreserving organized plant tissues, the problem of designing effective cryopreservation protocols for new or stress-sensitive plant materials remains. The ability of plant cells and organs to withstand the severe chilling, osmotic, and chemical stresses associated with cryopreservation varies significantly between species and even between genotypes. Therefore, adapting the cryopreservation procedure and cryoprotective solutions (CPAs) to new plant materials is an ongoing process. Furthermore, the traditional approach to vitrification methods, which involves preculture, osmoprotection, PVS2/PVS3 treatment, and unloading, is not always practical for wild species and sensitive plant materials. In this case, applying a conventional 'trial-and-error' approach, where each protocol step is adapted individually, is time-consuming and labour-intensive. It also requires a large amount of uniform starting material for experimentation.

The alternative systematic approach acknowledges the physiological complexity of plant tissues and the diverse range of behaviors observed during CPA treatment (Figure 1). Vegetative plant materials are classified into four categories according to their size and structure, as well as their response to osmotic and chemical stresses caused by CPA mixtures with different compositions and concentrations (Figure 2). Standard treatments with several

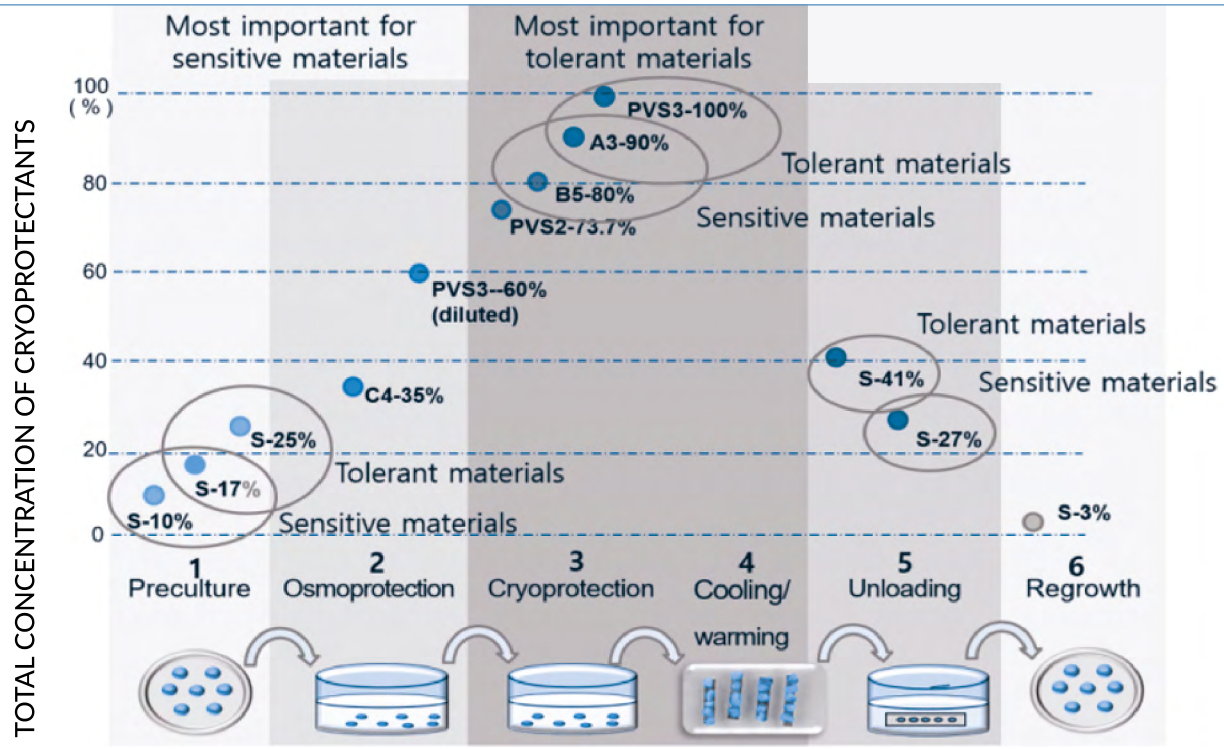

Figure 1. *Evolution of CPA concentrations in the course of a droplet-vitrification method and its importance based on material sensitivity (based on Kim and Popova 2023). S – sucrose. Solution compositions are given in Figure 2.*

modifications are designed for each category and can be applied to plant material to reveal its tolerance or sensitivity to osmotic and chemical stresses. The protocols that produce the best regrowth results are then combined to create the optimized procedure. In addition to the standard PVS2 and PVS3 solutions, modified osmoprotection and vitrification solutions are used in the treatment. This allows the treatments to be more flexible and adapted more precisely to the unique characteristics of each material type.

This approach uses a minimal amount of starting materials for the tests and provides a relatively accurate prediction of how the material will behave during cryopreservation and CPA-related stress. Over the past decade, this systematic approach has been successfully used to cryopreserve various plant materials, including bulbils, shoot tips, axillary buds, embryogenic tissues, and hairy and adventitious roots. The unifying principles revealed by this approach could broaden the spectrum of wild species and materials that can be safely conserved through cryopreservation.

A three-step regrowth process may improve the survival and regeneration of meristematic explants from particularly sensitive species after cryopreservation. The three steps include (1) three to five days on ammonium-free medium added with growth regulators in the dark to reduce the oxidative stress and improve survival; (2) approximately two weeks under dim light on a full-strength medium with growth regulators, to support cell division and the formation of new tissues; (3) a full-strength medium without growth regulators, to support the formation of normal plantlets. For growth regulators, a combination of low doses of a cytokinin and gibberellic acid seems to be the most effective.

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MATERIAL GROUP	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Examples of material types	Bulbil primordia 1.5–3.0 mm	Shoot tips, axillary buds 1.0–2.5 mm		Root apices 7–10 mm
Osmotic stress Chemical stress	Very tolerant Very tolerant	Sensitive Moderately tolerant	Moderately tolerant Sensitive	Very sensitive Very sensitive
Proposed standard cryopreservation protocols (bold letters) and their variations*				
Preculture	0.3 M (17 h) 0.5 M (17 h)	0.3 M suc (24 h or 48 h) 0.3 M suc (24 h) 0.5 M suc (17 h)		0.3 M suc (24 h) 0.3 M suc (48 h) 0.5 M (5 h)
Osmoprotection	C4–35% (50 min)	C4–35% (40 min)	C4–35% (30 min)	C4–35% (20 min)
Cryoprotection	**PVS3**–100% **(150 min)** A3–90% (150 min) 0°C	PVS3–100% (60 min) A3–80% (60 min) 0°C	B5–85% (60 min) A3–80% (60 min) 0°C	B5–80% (15 min) A3–70% (20 min) 0°C
Unloading	35% sucrose (50 min)	35% sucrose (40 min)	35% sucrose (30 min)	35% sucrose (20 min)
Regrowth	3–step regrowth starts with an ammonium-free medium with GA_3 + cytokinin followed by standard medium with GA_3 + cytokinin and then by growth regulator-free medium			

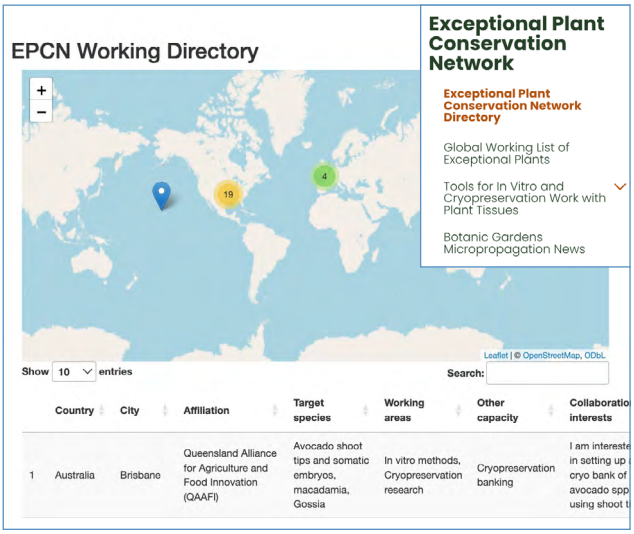
Figure 2. *Four categories of plant materials with tentative standard conditions (bold) and their variations at different steps of the droplet-vitrification method, which may be applied for initial testing of the tolerance/sensitivity of new plant materials (modified from Kim and Popova 2023). Composition of CPA mixtures (w/v): C4-35%: 17.5% glycerol + 17.5% sucrose; PVS3-100%: 50% glycerol + 50% sucrose; A3-90%: 37.5% glycerol + 15% DMSO + 15% EG + 22.5% sucrose; A3–80%: 33.3% glycerol + 13.3% DMSO + 13.3% EG + 20.1% sucrose; A3–70%: 29.2% glycerol + 11.7% DMSO + 11.7% EG + 17.4% sucrose; B5-80%: 40% glycerol + 40% sucrose.*

Tools for Exceptional Plant Researchers

Megan Philpott

Center for Conservation & Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, United States

The number of species considered exceptional is growing worldwide, but the capacity to conserve these species using specialized techniques like tissue culture and cryopreservation is not maintaining pace. In order to lower the barrier to entry in exceptional plant conservation and grow global capacity, the Cincinnati Zoo & Botanical Garden's Center for Conservation and Research of Endangered Wildlife (CREW) has developed a free online community, the Exceptional Plant Conservation Network (EPCN: cincinnatizoo.org/epcn), which hosts a number of online tools designed to make exceptional plant conservation more efficient. Included in this resource is the EPCN Working Directory, a searchable database of researchers around the world working in exceptional plant conservation, along with their research interests and capacities. This directory can facilitate collaborations between practitioners and serve as a resource for anyone looking to dig deeper into a particular species, group, or technique. There's also the List of Exceptional Status, a searchable database of over 23,000 unique species detailing their exceptionality status and the reasoning for their classification. This can quickly and easily allow researchers to search the potential seed storage feasibility of their species of interest. We also host tools designed to make plant tissue culture and cryopreservation protocol development easier and more efficient for practitioners. Resources in this category include the Plant Hormone Calculator, an application that allows researchers to quickly and easily convert common plant hormones from their concentration in mmoles (common in the literature) to mg/L (more common in media recipe development). The Comparative In Vitro Database is a repository of nearly 400 species and their somatic embryogenesis propagation protocols that researchers can use as a jumping-off point for protocol development or to compare average cytokinin and auxin types and amounts between related species. This resource is currently being expanded to include in vitro shoot propagation protocols and cryopreservation protocols in the future. The Media Comparison Tool allows researchers to compare formulas across over 30 commonly used plant tissue culture media, and includes comparisons across macro- and micronutrient ions and ions from iron. This can help inform protocol development by allowing researchers to compare and choose media with nutrient profiles appropriate to their target species. Finally, we have a searchable database of the Micropropagation News, a newsletter published by the Royal Botanic Gardens, Kew that contains a wealth of information on plant tissue culture protocols not found in the conventional academic literature. The database is searchable by species, topic, technique, and author, and unlocks a wealth of information previously hidden away in the gray literature. Using tools such as these, experienced and new practitioners of exceptional plant conservation can streamline their processes and make their own conservation work more efficient. In addition, as researchers contribute their own findings and data to these databases, we will be able to draw insights from across the wealth of species and techniques to elucidate the intricacies of plant tissue culture success.



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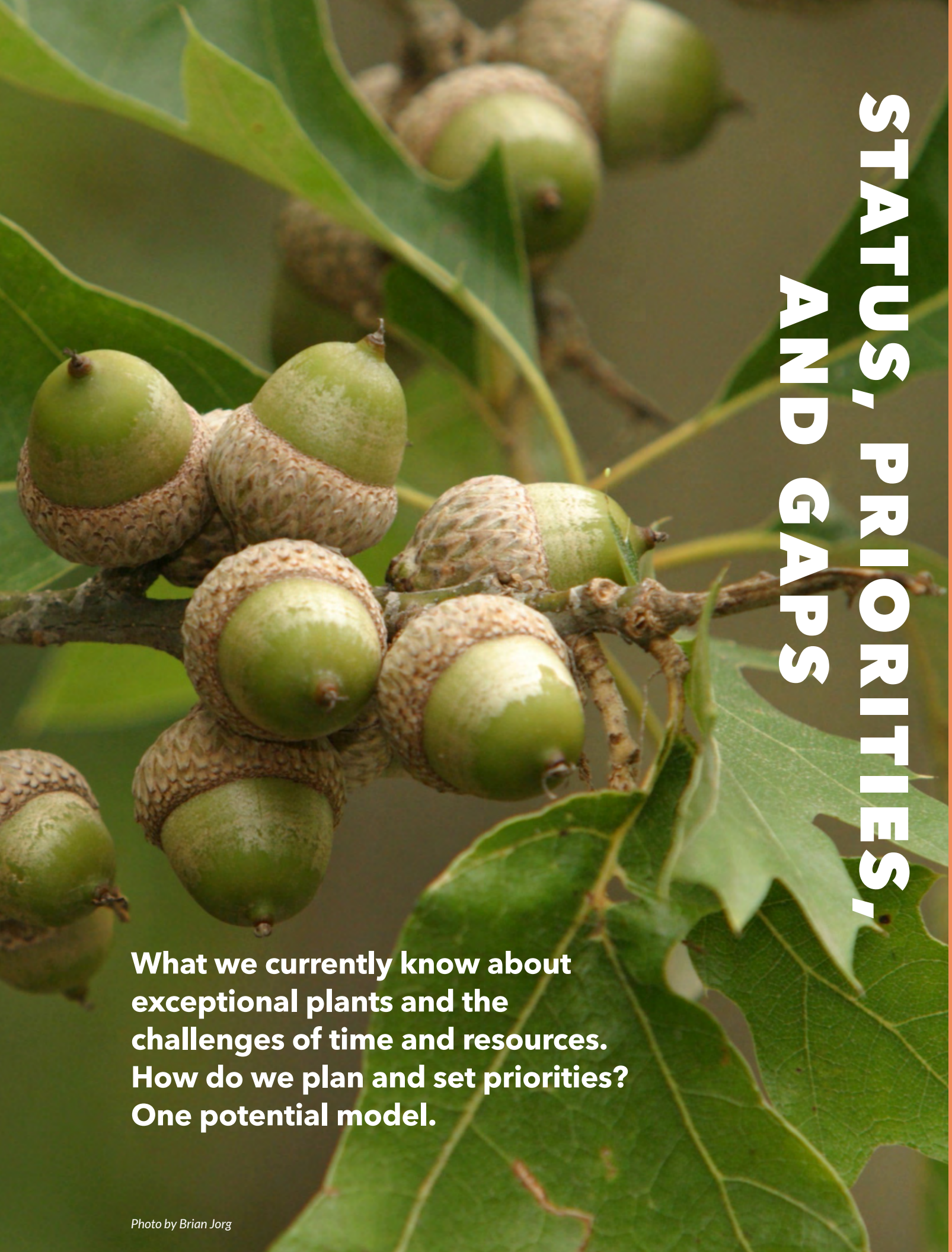

STATUS, PRIORITIES, AND GAPS

What we currently know about exceptional plants and the challenges of time and resources. How do we plan and set priorities? One potential model.

Photo by Brian Jorg

The Global Working List of Exceptional Plants

Valerie Pence

Center for Conservation & Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, United States

The list of exceptional plants was first published in 2022 in conjunction with a manuscript on a gap analysis of information on exceptional plants, but it now lives online at the Exceptional Plant Conservation Network website which can be found at http://cincinnatizoo.org/epcn. It was a compilation of information from the Seed Information Database from Kew, which now lives on the Society for Ecological Restoration website (https://ser-sid.org/), from the literature, and from expert comment, and 775 exceptional species were identified out of information on more than 23,000 species. The exceptional species were categorized, based on the roadblocks to conventional seed banking: 1) seeds not available; 2) seeds desiccation sensitive; 3) short-lived seeds; or 4) seeds deeply dormant. Other information was aligned with the species, including any updates in nomenclature, threat status and woodiness, if known, and any ex situ collections. Version 2 of the list has now been published, adding 262 exceptional species, largely from the Australian flora.

The goal of the list is to provide a tool for providing focus on species that require methods other than conventional seed banking and thus will require additional attention for their conservation. Many species on the list have not been investigated for the best approach to their long-term ex situ conservation. In addition, a list of 7,682 threatened congeners of the known exceptional species was also created (Tab B5 Supplementary data spreadsheet, https://www.sciencedirect.com/science/article/pii/S0006320721004912?via%3Dihub). It is known that seed storage characteristics, such as desiccation sensitivity or being short-lived, are often found in congeners, and thus threatened congeners of exceptional plants is a group that could help prioritize research.

This list is a start, but it represents less than 2% of the species predicted to be exceptional, so much more information is needed. An online form is available at https://czbg.jotform.com/form/92876073861165 to add information. The form is straightforward, asking for the scientific name, why the species might be exceptional, the source of the information (e.g. literature, personal observation, etc.), and if the species is known to be in an ex situ collection. It is hoped that seed researchers and seed storage practitioners can add their expertise to building the list. Other methods, such as predictive models, as outlined by Sommerville on page 36 should also be adopted to further broaden the list and provide a foundation for understanding the scope of the resources that will be needed for conserving exceptional species.

> "The List provides **A foundation for understanding** the scope of the resources that will be needed for conserving exceptional species."

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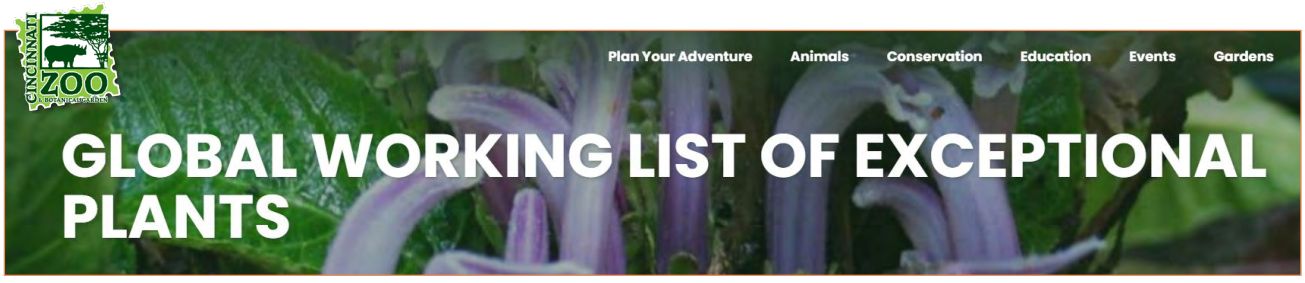

Conservation **Planning for Exceptional Plants** Species Summary

Yvette Harvey-Brown

Botanic Gardens Conservation International (BGCI), United Kingdom

There has been a huge increase on the number of tree species assessed on the IUCN Red List of Threatened Species, enabling the identification of species threats across their native range, and understanding which threats are having the biggest impact to trees. Understanding this is critical to conservation practitioners, who need to know what is threatened and why to prioritise their limited resources to implement strategic actions on-the-ground to save a species. Taking a step-by-step approach, following the IUCN Conservation Cycle, from assessments, to planning and then to action is the conservation model being increasingly used. However, many are uncertain about how to move from the assessment phase into conservation action. Therefore, conservation action planning is a necessary step to bring together partners across sectors to identify actions that will effectively mitigate the species threats and challenges long-term.

Following the IUCN SSC Conservation Planning Specialist Group (CPSG) principles and steps, Botanic Gardens Conservation International (BGCI) have been building momentum towards plant conservation action planning. In collaboration with CPSG, BGCI has facilitated conservation planning workshops in Kenya, Ghana, Chile and Malaysia. Thus, more conservation action plans have been developed for hundreds of priority, threatened tree species around the world. BGCI is now working with in-country partners to encourage implementation of these actions. Conservation planning provides an opportunity to identify the challenges associated with conserving exceptional species through participatory discussion and developing pragmatic strategies to address them. For each strategy, it is recommended to determine the actions, key stakeholders, timeline and resources required to implement.

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The Kenyan Threatened Tree Consortium has been established to implement the plan

Chillin'
The Global Plant Cryopreservation Initiative

David D. Ellis

International Potato Center (CIP), Peru

The Svalbard Global Seed Vault (SGSV) is an incredible advancement for the long-term secure safety back-up for plant genetic resources collections which can be maintained as botanical seed. Unfortunately, the genetic resources collections of five out of the ten most important crops for humanity (potato, cassava, banana, sweetpotato and yam) cannot be backed-up in the SGSV as they are conserved as clones and not botanical seed, as they are valued for unique allelic combinations in individuals which have been selected or bred to have an exceptional combination of desirable traits. In addition to crops which are vegetatively propagated, it is estimated that ~12% of plant species produce botanical seed which cannot survive at all (recalcitrant seed) or are short lived (<20 years) in the conditions of the SGSV. The genetic resources collections of these crops are all maintained in shorter-term conditions either in the field, green/glasshouses, or in vitro and all are at risk of loss without a long-term safety back-up strategy.

Based on recommendations from a 2017 *Feasibility Study for a Safety-Back-up Cryopreservation Facility* (Figure 1) that cryopreservation is the best long-term conservation option of clonal and recalcitrant seed genetic resources collections and that, *"A major global initiative is urgently needed to accelerate the development and implementation of cryopreservation"*, the Global Plant Cryopreservation Initiative (GPCI) was conceived. The GPCI seeks to provide a safety back-up system to protect this large and important group of irreplaceable genetic resources collections by providing, building, and supporting regional capacity for cryopreserving clonal, and recalcitrant and short-lived seed, genetic resources and to create a network of expertise for interested parties. It aims to ensure the long-term secure conservation for these collections and to maintain awareness for the need to back-up these collections before they vanish forever.

The GPCI will initially focus on the global south as this is where the majority of the most vulnerable and in need genetic resources collections exist. As stable funding and infrastructure is needed, the initial phase is being built around the International Agricultural Research Centers (IRACS) of the CGIAR. Cryopreservation "cryohubs" which will house the safety back-up cryobanks are being set up at the International Potato Center (CIP) in Lima, Peru, and the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. The hub in Latin America is currently a model for how the cryohubs will work as there is already a LAC plant genetic resources cryopreservation network established with over 15 countries currently participating and a new cryobank which will start serving as a back-up facility of national collections in 2015. Additionally, there is a cryohub at Bioversity International in Leuven, Belgium which supports, globally, the development of cryopreservation protocols and outreach for the GPCI.

"An approach where ***collections will be used for the betterment of human kind*** through a clean and share model"

The GPCI is a long-term vision for the future. The Initiative has demonstrated an approach where collections will be used for the betterment of human kind through a clean and share model and it is currently seeking funding to support continued operations and a secure food supply for the future.

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Figure 1. *The Feasibility Study for a Safety-Back-up Cryopreservation Facility (Acker et al. 2017) Available at https://cgspace.cgiar.org/handle/10568/91009.*

V-guadalupensis; Photo by Megan Philpott

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