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Cryopreservation: A Means to Store Plant Germplasm in a Small Space for a Long Time

Plant germplasm is the hereditary material from which new plants can be grown. It can be thought of as a "living genetic resource" maintained for plant breeding, preservation and research.[1] Germplasm is the fundamental component in all plant breeding programmes. The conservation of germplasm is critical for the development of future crop plants able to grow under changeable environmental conditions, use resources, such as water, more effectively, and produce more food for an increasing global population. The conservation of plant germplasm requires the preservation of genetic diversity, that is as many of the genes present in a particular crop plant as possible, and often any related species, so that it can be used at any time or place in the future.

Germplasm is often conserved "conventionally" in the form of seeds, which can be stored dry at ambient temperatures. However, conventional storage has many limitations, including short-lived seeds, seed-borne diseases, and the high costs associated with storage space and maintenance. While seeds stored at ambient temperatures often have a limited storage life, low seed moisture levels combined with storage at low (-18 °C to -20 °C) or ultra-low (-135 °C to -196 °C) temperatures lowers the metabolic activity of seeds so that they can remain viable for much longer. Two examples of global initiatives to conserve germplasm by storing dry seeds at low temperatures are the "The Millennium Seed Bank Partnership" (MSBP), which was formerly known as the Millennium Seed Bank Project, and the Svalbard Global Seed Vault. The MSBP is coordinated by the Royal Botanic Gardens, Kew, and was established as an "insurance policy" to help avoid the extinction of plants in the wild. Dry seeds are stored at -20 °C and the MSBP collection represents the world's largest collection of seeds from wild plant species. The Svalbard Global Seed Vault, some times referred to as "The Doomsday Vault," is located inside a mountain on an island in the Svalbard archipelago, which is halfway between mainland Norway and the North Pole. The aim of the Vault is to safeguard as much of the world's unique crop germplasm as possible, and the Vault has the potential to store up to 2.5 billion seeds at -18 °C. As of 24 November 2016 the Vault holds a diverse collection of 864,309 samples of seeds (https://www.croptrust.org/what-we-do/svalbard-global-seed-vault/), sourced from almost every country in the world. An overview of the samples held can been found on NordGen's public online database (http://www.nordgen.org/sgsv/).

While low temperature storage can be an effective means of storing germplasm the ultimate

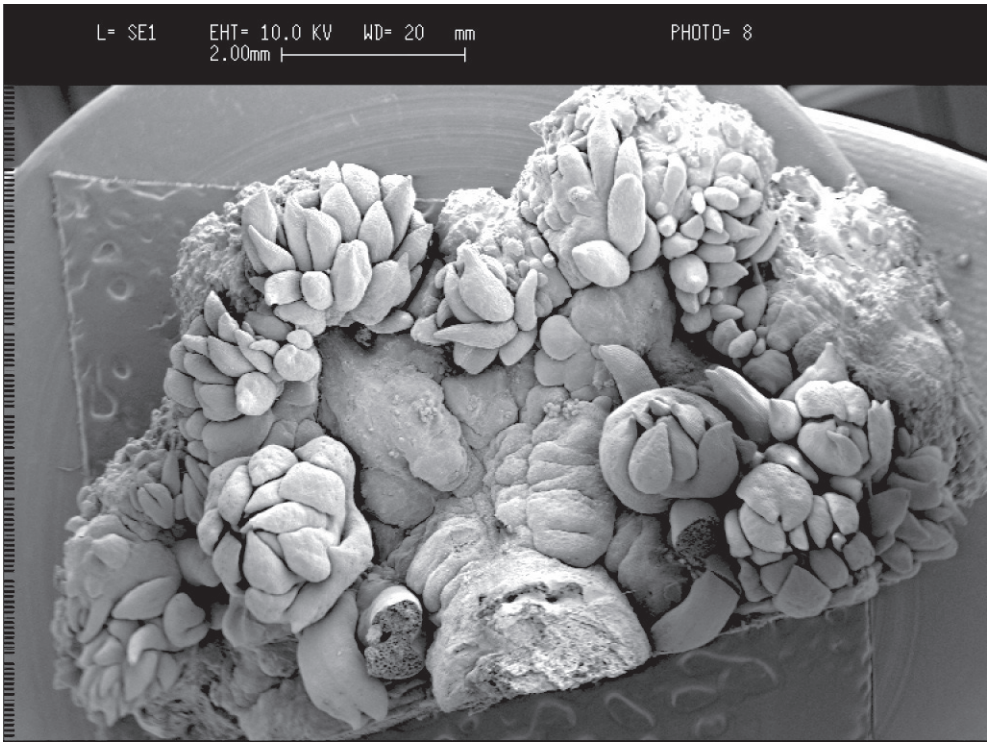

Figure 1. Pine (Pinus radiata) tree shoots regenerating in tissue culture. More than 20 shoots can be seen.

method for long-term storage, and for some plants, such as clonally propagated plants or plants that do not produce viable seeds, the only method for long-term storage in a relatively small space is cryopreservation. Cryopreservation is a versatile technique that involves the storage of germplasm, in the form of seeds, pollen, dormant buds, shoot tips, embryos, or isolated plant cells or tissues, in liquid nitrogen (LN) at or near to -196 °C, or in the vapour phase of LN at -135 °C.[2] Plant material is first preconditioned, using chemical and physical treatments, so that it remains viable when it is frozen and during ultra-low temperature storage. An important advantage of cryopreservation over ambient or low-temperature storage is that once cooled to LN temperatures viable samples can, in theory, be conserved indefinitely as no metabolic activity occurs at these ultra-low temperatures. Following re-warming, seeds and embryos can germinate, buds or shoot tips can be induced to grow, and whole plants can be regenerated from cryopreserved cells or tissues, using cell and tissue culture techniques.[2] These plant cell and tissue culture techniques also enable large numbers of clonal plants to be produced from very small tissue samples, as can be seen in Figures 1 and 2.

The science of plant cryopreservation began with the freezing of mulberry twigs in LN in 1965.[3] Since then, methods for the cryopreservation of numerous plant species have been developed and are now being adapted to cryopreserve a wide range of plant species.[4] Today important crops, such as wheat, potato and various fruit and forest trees, can be cryopreserved, rewarmed and then allowed to grow into complete plants.[5]

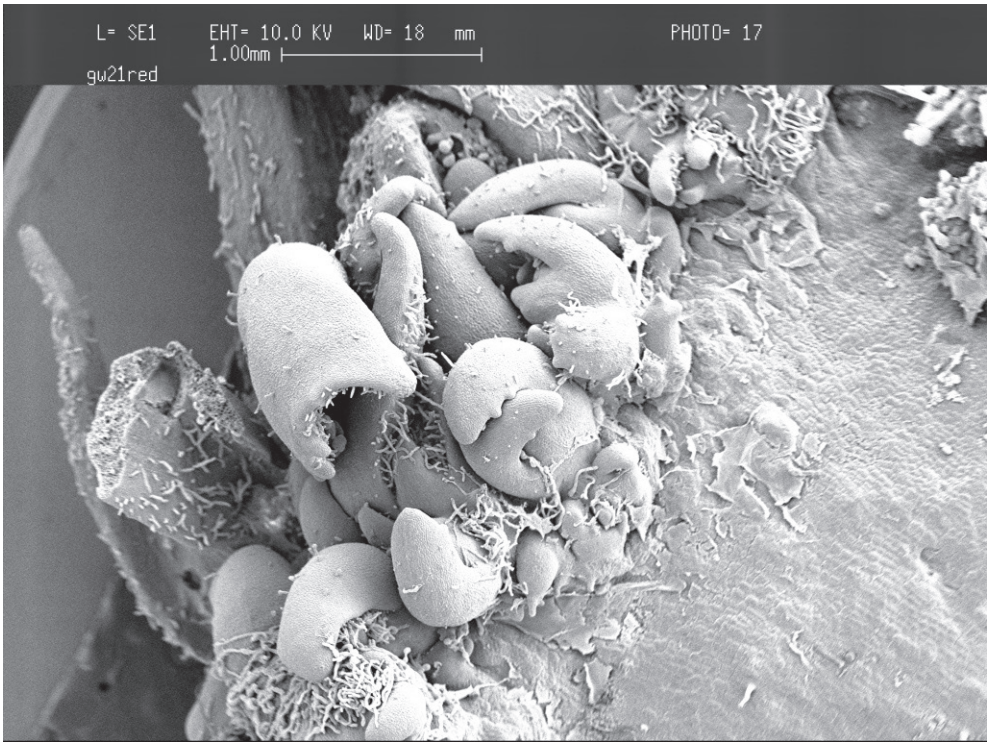

Figure 2. Lettuce (Lactuca sativa) shoots regenerating in tissue culture. Multiple shoots can be produced from a 1 mm square tissue sample.

Unlike conventional seed or plant collections, which require maintenance, the only cost associated with cryostorage of plant materials, often called "cryobanking," is the addition to the storage vessels of a small amount of LN each week. Cryobanking is now becoming a very cost-effective method for the long-term conservation of plant genetic resources, enabling the extended storage of large amounts of genetically diverse plant material, in a relatively small space, for extended periods of time. For example, a medium-size storage vessel can store 5,000-10,000 individual plant samples in LN.

A further advantage of cryopreservation over conventional storage techniques is that once in cryogenic storage there is virtually no risk of new microbial contamination and the development of disease. In fact "cryotherapy" has been shown to be an effective tool to produce virus-, phytoplasm- and bacterium-free plants. Cryotherapy can be used to produce disease free plants from diseased stock plants and can eliminate the risk of seed-borne disease, and hence reduce the obstacles associated with the transport of plant germplasm through national and international borders.[6]

Recently there has been a resurgence of interest in growing plants in space, as sources of food and oxygen for long duration space flights. For example, NASA plant researchers are exploring the "Question of Deep-Space Food Crops."[7] While still in the realms of science fiction, the potential for cryopreservation to enable the storage of large amounts of genetically diverse plant material, in a relatively small space, for an extended period of time, might mean that plant cryobanks could be

very useful for the transportation of plant germplasm as part of the colonisation of outer space by humans.

David Burritt's research is focused on answering fundamental questions associated with how organisms survive under environmental stress and when exposed to toxins and other pollutants, both of which are increasing global problems. Much of his research has applications in the biotechnology, crop, food and biomedical industries. He is an Associate Professor in the Department of Botany at the University of Otago.

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