PLANTS PEOPLE
POSSIBILITIES

Plant cryopreservation

What is cryopreservation? The word cryo- comes from the Greek for ice, frost or cold. Preserve means 'to keep safe from harm or loss'. Cryopreservation is the process of freezing living material to keep it safe. This is normally done at or near the temperature of liquid nitrogen, -196°C (-320°F). At this temperature physical and metabolic cellular processes are effectively stopped and the living tissue is in a state of suspended animation, free from pathogens and the risk of genetic drift. In theory, plant tissue can be stored in this way indefinitely. When required, it can be recovered and grown on to regenerate a whole plant.

Cryopreservation is a valuable technology for the cost-effective, sustained long-term conservation of plant genetic material. A variety of plant material can be used, including cells in tissue culture, pollen, seeds or parts of seeds, embryos, tissues from the early stages of development of mosses and ferns, buds, twigs, and meristematic (growing point) tissue.

Conservation

The best and most cost-effective way of protecting plants and animals is *in situ* – in their natural habitat – for example in national parks or wildlife sanctuaries. However, it is often necessary to conserve many rare and endangered species *ex situ* – outside their natural habitat – in institutions such as botanic gardens, zoos, and aquaria. The Royal Botanic Gardens, Kew plays an important international role in maintaining a variety of unique and rare species, many of which face the imminent threat of extinction. One of the simplest ways to conserve plants *ex situ* is by seed. This is because seeds allow you to store more genetic diversity in a small space at low cost.

However, some plants produce seed that cannot be dried or stored at low temperatures. There are also plants that cannot produce sufficient or viable seeds, that exist now only as male or female specimens, produce seeds infrequently, or are extremely rare. In these cases the plants can be conserved in planted collections, in tissue culture or by cryopreservation. Of these, cryopreservation demands the least space and reduces susceptibility to disease, mutation and environmental conditions.

The role of cryopreservation at Kew

At Kew, we have been researching methods for the cryopreservation of vegetative material of rare and endangered species since 1992.

Part of this research involves developing methods to cryopreserve tissue from those plants which cannot be stored as seed. Each individual species reacts differently to cryopreservation and so a different protocol needs to be developed for each species put into cryo-storage. Once a protocol has been developed, cryopreservation offers an effective, inexpensive and secure method, which is independent of continued electrical supply, for the long-term conservation of plants.

The Micropropagation Unit at Kew has developed techniques for the cryopreservation of many ferns, mosses, orchids, shrubs and herbaceous plants.

The process simplified

Each species has a unique protocol, but in general the plant is put into tissue culture. The part to be used is isolated and treated with chemicals that help to reduce the adverse effects of freezing and/or dehydration. Examples of these chemicals include glycerol and concentrated sugar solutions. Tolerance to cold treatment may also, in some instances, be increased by exposing the tissue to low temperatures or plant hormones before it is frozen. The water content of the cells is then lowered and the plant tissue frozen and stored in liquid nitrogen. When needed the tissue is carefully thawed, and regrown under controlled conditions to produce a new plant.

Interesting cryogenic facts

- Liquid nitrogen is made by the fractional distillation of liquefied air.
- Scientists have been considering the concept of freezing tissue to 'maintain' life since the 1700s. However, the first reported successful use of cryopreservation was in 1949 for animal cells. It was not until the 1970s that plant cells were preserved.
- Scientists believe that cells can be stored in liquid nitrogen for hundreds to thousands of years and still be revived.
- Some cryopreserved cells have been successfully revived after more than half a century.
- Plant cryopreservation is based on the principle of totipotency: that any living cell, provided it has not become too specialised, has the inherent capacity to regenerate an entire plant.
- Cryopreservation is now routinely used to store reproductive cells from animals and humans for artificial insemination.
- Human tissue banks often store cells and tissues such as ovarian tissue, blood cells, and skin biopsies. These can be used for medical procedures, rederivation of storage material and screening for infectious pathogens.
- Some companies will cryogenically freeze human bodies on the pretext that when the technology is developed one will be brought back to life in the future (at an initial cost of about £80,000).
- The lowest temperature ever achieved is just above absolute zero, -273oC (-459oF). Infrared lasers were used to reduce the energy of caesium atoms in a vacuum.
- Cryogenic treatment of plastics and metals is believed to re-align and compact their molecular structure giving them improved strength and other physical properties. For this reason every vessel sent into outer space is cryogenically treated in an effort to reduce the wear and tear effects of friction and heat.

Further information

For further information on our work or specific enquiries about particular plants or techniques please contact:

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Cryopreservation techniques have been investigated for many species including (pictured left to right): *Lepisorus longifolia, Medusagyne oppositifolia, Paralophia epiphytica, Pteris adscensionis* and *Thunia alba*

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