Longevity-extending methods for perishable seeds

Latent Life

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Mighty, robust trees like the chestnut or oak in fact produce surprisingly short-lived seeds. Fortunately, modern cryogenic methods can help prolong their conservation

Plants of the gymnosperm and angiosperm varieties ensure the continuation of their species by producing seeds. Once mature and fully developed, such seeds carry the latent spark of life and can give rise to new generations. Although plant seeds are commonly thought to retain this life-starting ability for a very long time, species vary greatly in this regard: while many plants' seeds do retain the ability to sprout for decades, some species' seeds only remain capable of producing seedlings for a very short duration (from several weeks up to several months under optimal conditions). In order to ensure the better preservation of Poland's domestic species, we are attempting to develop effective storage methods to boost the longevity of such plant seeds.

Chilly vapor

Whether a given seed maintains or loses its vitality as time passes chiefly hinges upon its sensitivity to dehydration. Anyone who has ever gathered and brought home chestnut seeds and then tried to pot them even just a few days later can attest to this: such efforts most likely ended in failure, as the dried-out seeds were already dead by the time they were planted. Yet seeds of other trees, such as the Scotch pine (*Pinus sylvestris* L.) or Norway maple (*Acer platanoides* L.) stored in similar conditions retain their vitality much longer.

One technique for amassing economic reserves of plant seeds or conserving genetic resources involves storage at a temperature of -10° to -20°C - but since resistance to dehydration varies so greatly this technique leads to the destruction of certain species' seeds. Nonetheless, more advanced cryogenic conservation techniques involving the storage of plant tissues at the boiling temperature of liquid nitrogen (-196°C) or its vapor (around -135°C) have seen rapid progress since the late 20th century. Such advances have yielded prospects for successfully preserving seeds of

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Depending on the species, tree seeds can retain their longevity from as little as several days up to a decade or more. Here (from bottom to top): nuts of the silver birch with husks, nuts of the common alder, spherical seeds of the small-leaved lime, winged nuts of the mountain elm, nuts of the European hornbeam, pits of the wild cherry, triangular nuts of the common beech, and samaras of the common ash

the problematic or *recalcitrant* class, with relatively short longevity (e.g. the chestnut, oak, or sycamore). Here the freezing technique is applied to seed germs that are first isolated from whole seeds, or to the even smaller embryo axes. Research has shown that germs or embryo axes can survive drying down to 18-33% moisture content, whereas whole seeds can bear drying to 12-50% moisture content (depending on the species). Isolated seed fragments can then be subjected to techniques that prevent the formation of ice crystals, which are harmful to cell life. This procedure, called "cryoprotection," facilitates the safe, amorphous solidification (vitrification) of cytoplasm. Usually it requires the presence of protective substances like sucrose, glycerine, polyethylene glycol, or DMSO (dimethane sulphoxide) and very rapid freezing, achieved through direct tissue contact with liquid nitrogen. Tissue activity is later recovered through rapid thawing at a temperature of 40°C.

Sensitive as an oak?

At the PAN Institute of Dendrology in Kórnik, we have investigated the potential to successfully freeze and store embryo axes isolated from the acorns of the English oak (*Quercus robur* L.). This tree is of the *recalcitrant* class - whole oak seeds retain their vitality for a very short duration, thus precluding their preservation in traditional gene banks.

Satisfactory effects were achieved when embryo axes were first subjected to cryoprotection in sucrose and then glycerine solutions. Next the germinal tissue was dried down to 25% moisture content - the point at which embryo axes begin to slowly lose vitality as a consequence of dehydration, but at the same time demonstrate the highest post-thaw survival rate. By way of comparison, whole acorns begin to lose vitality at around 35-38% moisture content, and when frozen die at a temperature of -6° to -8°C. Our embryo axes thawed from -196°C were cultivated *in vitro* in an agar medium to produce properly growing seedlings, which have now grown to small trees in the Institute of Dendrology's experimental forest.

Our research likewise investigated another approach: using liquid nitrogen to freeze not seed fragments, but rather embryogenic callus tissue of the English oak previously obtained from *in vitro* cultivation of immature germs. This tissue consists of rapidly dividing cells that constitute the beginning of future somatic germs, which can be used to produce a whole plant if the right culture mediums are used. Our attempts were successful - germs were produced from frozen embriogenic callus in *in vitro* cultures, used to cultivate correctly growing seedlings and subsequently whole English oak trees.

"For future centuries"

The PAN Institute of Dendrology in Kórnik is also studying the sensitivity of seeds from categories more resistant to drying, called the *intermediate* and *orthodox* classes. We have managed to identify the safe moisture-content limits for the liquid nitrogen freezing of seeds from more than 20 types of forest trees. The success of our experiments allows us to conclude that the longevity of frozen seeds is significantly longer than that of seeds preserved in classical conditions. Similar conclusions are to be drawn from the research of other groups. Researchers from the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado, have extrapolated the results of 30-year storage (so far the world's longest) of seeds in liquid nitrogen or its vapor - showing that the estimated storage lifespan should be calculated in hundreds of years, rather than decades!

In the wake of the ecological disaster that took place in the Izera Mountains in the 1980s, the modern Kostrzyca Forest Gene Bank was set up in 1995. The center presently preserves plant gene resources in classical conditions, but since 2005 it has likewise been working with cryoconservation techniques. The genetic resources of threatened species will be amassed there, stored away in liquid nitrogen, enabling contemporary forest flora to be preserved for future generations as frozen sparks of life. ■

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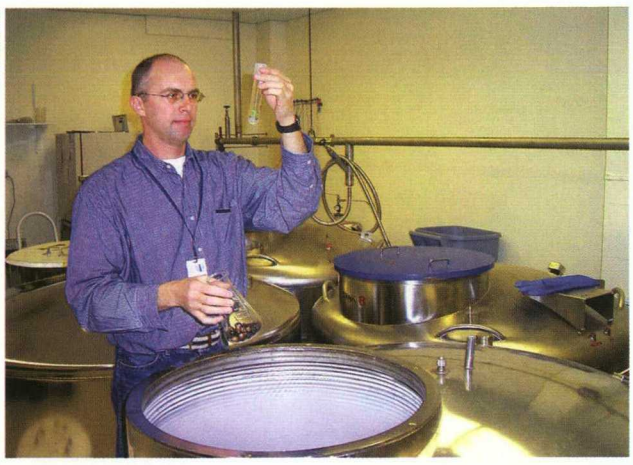

Storing seeds or isolated seed tissues in liquid nitrogen vapor (-135°C) is a technique that allows plant genetic resources to be preserved for as long as hundreds of years. The author of the present article at the National Center for Genetic Resources Preservation in Fort Collins, Colorado

Further reading:

Suszka B., Chmielarz P., Walkenhorst R. (2005). How long can seeds of Norway spruce (*Picea abies* (L.) Karst.) be stored? *Annals of Forest Science, 62*, 73-78.

Walters C., Wheeler L., Stanwood P. (2004). Longevity of cryogenically stored seeds. *Cryobiology, 48*, 229-244.

Suszka B., Muller C., Bonnet-Masimbert M. (1996). *Seeds of forest broadleaves from harvest to sowing* (pp. 1-194). Paris: INRA.