

The selected findings presented here are innovative in terms of their applications, and after further development or refinement they may be harnessed in industry or the services sector. The uses of these achievements may indeed be multiple: they may be implemented by industry as new products or as new research methods for evaluating the quality of products or services.

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SELECTED RESEARCH FINDINGS OF AN INNOVATIVE NATURE



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POLISH UNIVERSITIES
AND THE INSTITUTES
OF THE POLISH ACADEMY
OF SCIENCES

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Cryopreservation of genetic diversity of endangered forest tree species producing difficult-to-store seeds

■ OVERVIEW OF RESEARCH FINDINGS

Cryogenic conservation techniques involve the storage of plant tissues at the boiling temperature of liquid nitrogen (-196°C) or its vapor (around -135°C) and very often use an *in vitro* technique to recover whole plants from cryopreserved explants.

The Institute of Dendrology of the Polish Academy of Sciences in Kórnik has developed successful cryopreservation techniques and cryopreserved the genetic diversity of the pedunculate oak (*Quercus robur* L.), an endangered species in Europe. This is the first experiment described in the world literature involving the cryopreservation of *Quercus robur* embryonic axes used to obtain plants. At present, small trees derived from embryonic axes frozen in liquid nitrogen are growing in the Institute's experimental forest.

The acorns of the pedunculate oak belong to the recalcitrant seed category, meaning that they are very sensitive to desiccation and low temperatures (below -10°C), making them very difficult to keep alive over a relatively short storage duration. In the course of the experiment, several millimeter-long embryonic axes isolated from acorns were first subjected to

cryoprotection in sucrose and then glycerol solutions. Before they were frozen in liquid nitrogen, embryonic axes were dried down in control conditions to 25% water content, thawed from -196°C at 40°C , and then cultivated *in vitro* on the woody plant agar medium to produce properly growing seedlings (Fig. 1).

Apart from recalcitrant seeds, researchers at the Institute also investigate the sensitivity of seeds from categories more resistant to drying, i.e. the intermediate and orthodox classes (Fig. 2). They have man-

Fig. 2. From bottom: nuts of a common birch with husks, nuts of a black alder, spherical seeds of a small-leaved linden, winged nuts of a mountain elm, nuts of a European hornbeam, pits of a bird cherry, triangular nuts of a European beech, and samaras of a European ash

Fig. 1. Pedunculate oak *in vitro* plantlets, obtained from embryonic axes frozen in liquid nitrogen



aged to identify safe moisture content limits for the freezing of seeds of more than 20 forest tree species. Both broadleaves and coniferous seeds of forest species were stored in liquid nitrogen at the Institute's laboratory for two years. The success of these experiments leads to the conclusion that the longevity of frozen seeds is significantly longer than that of seeds preserved in standard conditions. Similar conclusions are to be drawn from the research of other groups. For instance, researchers at the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado (with which the Institute of Dendrology collaborates, Fig. 3) extrapolated the results of 30-year storage (so far the longest in the world) of orthodox seeds in liquid nitrogen or its vapour – show-

ing that the estimated storage lifespan should be reckoned in hundreds of years, rather than decades.

The results of the Institute's research have been used in the Kostrzyca Forest Gene Bank (<http://www.lbg.jgora.pl>) established in 1995 in the southern part of Poland, where an ecological disaster took place in the 1980s. The center presently preserves plant gene resources in standard conditions and it boasts a cryogenic unit (Fig. 4) created in 2007. In times of global climatic change, ecological disasters and fires, cryopreservation techniques will enable the most valuable gene resources of endangered Polish forest flora, which can not be stored by classical methods, to be safeguarded for the future.



Fig. 3. Storing seeds or isolated seed tissues in liquid nitrogen vapor (-135°C) is a technique enabling plant genetic resources to be preserved for even as long as hundreds of years. Paweł Chmielarz (Institute of Dendrology, Polish Academy of Sciences) develops methods to cryopreserve oak germplasm within the framework of a project funded by a competitive grant from the Kosciuszko Foundation at the National Center for Genetic Resources Preservation in Fort Collins, Colorado.



Fig. 4. The Cryogenic Laboratory at the Kostrzyca Forest Gene Bank, created in 2007 (Poland)

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