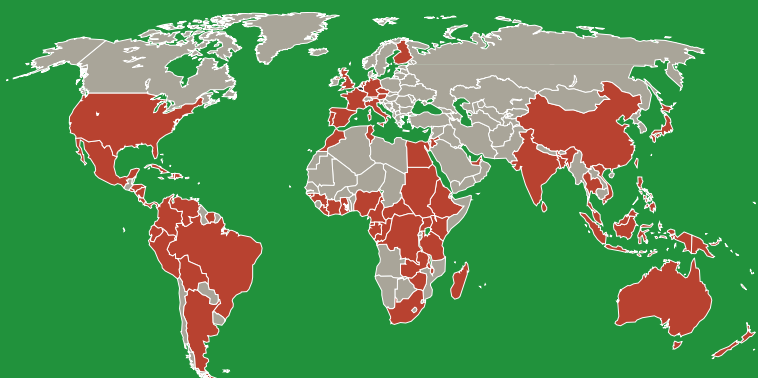


# High-tech care



Since its creation in 1985, the ITC has shipped more than 60 000 *Musa* samples to 200 institutions in 88 countries.

The fact that Belgium does not grow bananas played in its favour when, in 1985, the newly-created INIBAP opted for the university town of Leuven as the place to set up the INIBAP Transit Centre (ITC). As the name suggests, the genebank was meant to be a hub for germplasm movement. Locating it in a non-producing country facilitated the receipt of banana samples from anywhere in the world, due to the absence of quarantine restrictions. Distributing it, however, was another matter. Care had to be taken not to pass diseases along with the plant material, especially when the destination was a place still exempt from certain diseases.

Because cultivated varieties of bananas are seedless, they are conserved as plantlets in test tubes.

Since domesticated bananas do not produce seeds, scientists have used the plant's propensity for producing shoots to conserve bananas as small plantlets in test tubes. Because they are grown on a sterile medium, it is possible to ensure, through rigorous testing and culture procedures, that they are free of bacteria and fungi. This is not necessarily the case for viruses, which—as cell parasites—may need special therapy and intensive testing (carried out by certified 'indexing' centres) to be reasonably confident that the plantlets are virus-free and can be cleared for distribution. Indeed, the continued presence of viruses in 37% of the accessions means that this part of the collection is not available for general circulation, although therapies have recently been developed for some viruses and research is continuing to find ways of removing the others.



Deep-freezing meristematic tissue to the temperature of liquid nitrogen (-196°C) makes it possible to store viable plant tissues for thousands of years. B. Panis, KULeuven

Even though the temperature and lighting are kept at a minimum to slow down the growth process, the plantlets eventually outgrow their test tube. As a consequence, each accession is re-cultured once a year. In addition, experience suggests that tissue-culture material should be replaced every 10 years to control for the risks of somaclonal variation, altered characteristics in plant tissues that have been kept in test tubes for an extended period of time. A programme to rejuvenate the collection was launched in 2001. For a given accession, plants are grown in greenhouses and 'decapitated' to supply suckers. From these suckers is derived material both to replace the tissue-culture samples in medium-term storage, and for sending into the field so that taxonomists familiar with the particular material can check for its trueness-to-type.

Research carried out at KULeuven has provided an extra level of insurance to the conservation effort by developing methods that allow all kinds of banana to be safely conserved in liquid nitrogen. At these ultra-low temperatures, so-called 'cryopreservation' arrests both the growth of plant cells and all processes of biological deterioration, so that the material can be preserved, safely and cost-effectively, and resuscitated into fully viable banana plants. So far, more than one-third of the banana collection has been safely stored away in liquid nitrogen and, as yet further insurance, a duplicate set is being prepared for safe-keeping at a separate location. The expertise developed in the process has led to the recognition of KULeuven as a centre of excellence in cryopreservation, not just for banana but for other crops as well.

## ONE-SIZE-FITS-ALL CRYOPRESERVATION

As recently as three years ago, scientists trying to cryopreserve parts of plants would try something and if that didn't work, they would try something slightly different. This trial-and-error approach produced results but was not efficient when venturing into unexplored territory. A European project—CRYMCEPT, short for establishing CRYopreservation Methods for Conserving European PlanT germplasm collections—changed this when it set out to identify the crucial components needed for successful cryopreservation. The scientists from the Laboratory of Tropical Crop Improvement at KULeuven were part of a multi-national team of scientists working on different plants and analysing various parameters.

Contrary to what might be believed, the main key to success is not having plant tissues that are tolerant to freezing—otherwise, being tropical, banana plants could not be cryopreserved—but in having plant tissues that are tolerant to dehydration. The major concern when freezing living cells is avoiding the formation of ice crystals. Ice crystals are capable of puncturing the cellular membrane, which will then lose its capacity to control what goes in and out of the cell. Avoiding ice crystals by removing all the water is not an option, as the cell will surely die. The solution, known for some time, is vitrification, a solidification process that does not involve the formation of ice crystals and gives the tissue a glassy look.

The KULeuven researchers were already using vitrification to cryopreserve banana meristems. Prior to freezing, the cells are exposed to a vitrification solution high in compounds like sucrose and glycerol. Osmosis causes some of the water to move out of the cells into the solution, dehydrating the cells without killing them.

The vital new ingredient was to add a drop of the vitrification solution to a section of meristematic tissue placed on a small piece of aluminium foil and then plunging it into liquid nitrogen. Being syrupy, the solution sticks to the foil. Compared to the standard procedure, in which the tissues are placed in a small tube, this method more quickly freezes the tissues, which are now in direct contact with the liquid nitrogen (it is only after they have been frozen that the plant cells are stored in a tube). Rapid freezing and thawing turned out to greatly increase survival when the tissue is subsequently thawed.

After succeeding with bananas, the KULeuven scientists tried their cryopreservation protocol with taro, potato, strawberry, chicory, date palm and pelargonium. Their method worked almost immediately. When they have had to make adjustments, it was often in the part of the tissue-culture plant that was used or in the amount of time the meristematic cells are exposed to the vitrification solution.