

Banana diversity and proteomics in Leuven

The Good and the Bad

Banana cultivars are conserved longterm by freezing their germplasms in liquid nitrogen. However, some endure the procedure much better than others. Bart Panis and his team try to understand why – by using proteomics.

“Why is the banana bent?”

Each child ponders this ordinary question. However, scientists also ask banana questions.

Bart Panis, for example, from the Lab-

oratory of Tropical Crop Improvement at the Catholic

University of Leuven, Belgium, does – but he is not interested in the fruits' shape.

Of course, Panis knows that not all bananas look like the ones we are familiar with in Europe. “The fruit we buy in our supermarkets is the “Cavendish” dessert banana”, the agricultural engineer explains but this is only one of the numerous different banana (*Musa* spp.) varieties existing in the world. “Our lab houses the world's *in vitro* collection of banana germplasm”. This “International Network for the Improvement of Banana and Plantain (INIBAP) Transit Centre” counts about 1200 accessions of banana cultivars. A great biodiversity the researchers want to keep as safe as possible, not only for species protection.

Error-prone standard procedure

“In a lot of developing countries in Africa, Latin America and Asia, the banana is one of the most important crops!” Bart Panis points out. On the one hand, for these countries of the tropics and subtropics, the banana is an important commodity on the local and international markets. On the other hand, for millions of people, the banana – in that case the plantain or cooking banana – belongs to essential daily food! Many diseases can attack this important plant – a plant that incidentally is not a tree but an herb.

Therefore, the Belgian scientists are working to conserve the variety of cultivars and thereby ensure effective banana breeding.

Most banana cultivars cannot be conserved in a conventional seed gene bank because they do not have seeds. “At the moment, the plants are preserved *in vitro*,” says Bart Panis. That means as small glass tube plants under slow growth conditions with low temperature and light intensity. “However, this material still grows, so it needs to be sub-cultured, at least once a year”, says Panis and explains the disadvantage of this method: “It is quite labour intensive and due to contamination or human error plants can be lost.”

Harmful water

How can we improve the procedure of banana germplasm conservation? Bart Panis and his team from the Plant Physiology group in Leuven investigate this question. The 43 year old is committed to cryopreservation as the method of choice to store biological material long term. Once the material is transferred to ultra-low temperatures, like the -196°C of liquid nitrogen, it can be stored for almost unlimited periods! This has been done with animal and human tissues so why not with banana meristems.

The plant tissue consists of undifferentiated cells, where growth takes place and from which complete plants can emanate.

Of course, the difficulty is for something to survive in liquid nitrogen! The main problem you have to avoid is intracellular ice crystal formation that usually takes place under these freezing conditions and then kills the cells. “This is the bottleneck for successful cryopreservation,” says Bart Panis.

Enter proteomics

To overcome this, the scientists remove a lot of water from the meristems – but at the same time, they generate osmotic stress. “To dehydrate the tissues we pre-culture them on high sucrose media, thus the water is withdrawn osmotically”, explains Sebastien Carpentier, PhD a student in Bart Panis' group. Some banana cultivars endure this treatment quite well; they are dehydrated – and thereby cryopreservation-resistant, but not all! “We can differentiate good and bad responders”, says Bart Panis. So far, about 600 banana cultivars have been stored in liquid nitrogen.

To understand which molecular players might be responsible for the acclimation of the well-responding cultivars and what happens during the high osmotic stress treatment, Carpentier, Panis and Co. started to analyze the proteome of the meristems.

Glycolysis and cell wall integrity

Recently, they identified a list of proteins that obviously influence the bananas' reaction to osmotic stress and dehydration (*Proteomics* 7: p.92). “In the dehydration-tolerant variety we found proteins involved in the energy metabolism, for example, especially the ATP generation, to be up-regulated,” describes Sebastien Carpentier, who analysed the proteome of the so-called cauliflower-like banana meristems. “Under stress situations, cells have a higher energy demand; thus, the induction of genes for energy-conserving glycolysis seems to be essential for survival during osmotic treatment. In addition, it enhanced the amount of proteins responsible for cell wall integrity. On the other hand, after high sucrose exposure, we found the intracellular storage proteins down regulated, indicating that to



Sebastien Carpentier (l.) and Bart Panis



Banana meristem, before freezing

act as an amino acid source they may be too degraded."

When comparing the tolerant cultivar "Cachaco" with the dehydration-sensitive "Mbwazirume" the researchers discovered several genotype-specific protein isoforms and proteins that responded differently to high osmotic conditions.

"With these results, we know some of the players which enable the good responders to survive cryopreservation." Bart Panis is confident. "This is a step in the right direction!" Nevertheless, he knows, "These are some but not all members of the essential pathways we have identified." In the next step, he wants to find the missing players and illuminate the role of the identified protein isoforms.

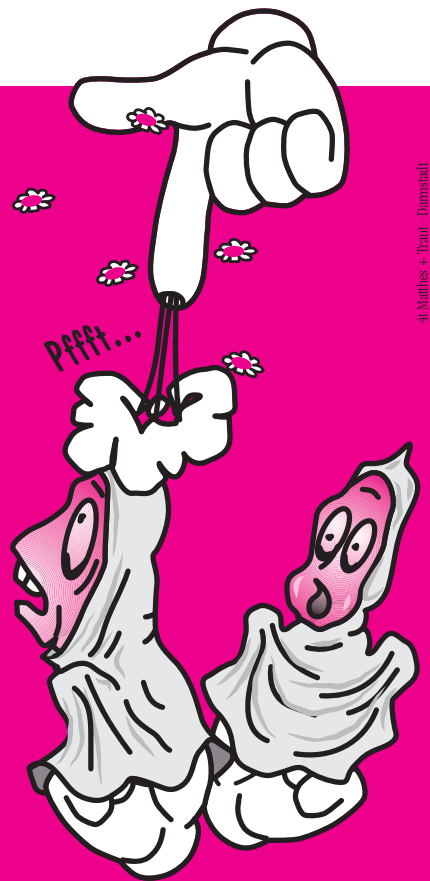
Towards the universal protocol

The long-term objective the Belgian researchers are focussing on is obvious. "If we can fully identify the molecular differences between the different varieties, we might finally be able to improve our cryopreservation protocol for all banana cultivars." Maybe then, Bart Panis hopes, banana-producing farmers in Africa and Latin America can use the information to advance the breeding.

SUSANNE DORN

One fine day in the lab...

by Leonid Schneider



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