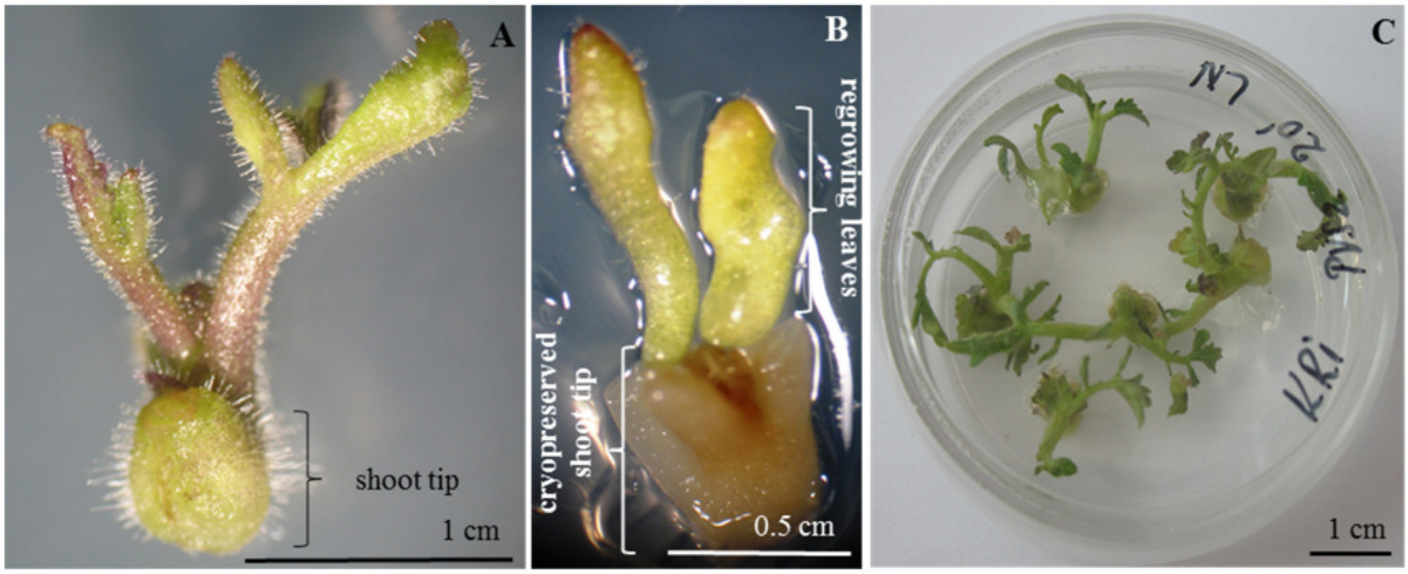
Surface-enhanced Raman spectroscopy of genomic DNA from in vitro grown tomato

Conservation of plant genetic resources is necessary for food security and to safeguard biodiversity which is essential for genetic engineering and plant breeding programmes. Conservation of plant resources can be achieved either in their natural habitats (*in situ*) or outside their natural habitats (*ex situ*) and both systems have been implemented for various plant species. For species which do not produce seeds or are vegetatively propagated, conservation in seed banks is not appropriate and therefore the most common method of preserving the genetic resources of these species is as plants in the field. There are, however, several problems with field gene banks, such as exposure to diseases, pest attacks, natural hazards and high costs for preserving the collections. To avoid this problems alternative conservation methods have been developed. Cryopreservation of plant genetic resources is a long-term storage method of plant material (such as shoot apices, zygotic and somatic embryos, cell cultures and pollen, etc.) at ultra low temperatures, in liquid nitrogen (-196°C) or in its vapor phase (-150°C) in such a way that the viability of stored material is maintained following rewarming. Storage at these temperatures is presumed to provide an indefinite longevity to the cells because the metabolic processes are retained. For successful cryopreservation, it is essential to avoid intracellular freezing and to induce the vitrification state of plant cells during cooling in liquid nitrogen and, as a result different cryopreservation procedures have been developed. Ensuring the genetic integrity of preserved plant material is of significant importance for quality germplasm conservation and its sustainable use. It is important to underline the complementarity of cryopreservation with the traditional conservation methods in plant biodiversity conservation actions.



(A) Non-cryopreserved (control) tomato shoot tip with leaves
(B) Cryopreserved shoot tip with regrowing leaves

(C) Tomato plants grown from cryopreserved shoot tips

In this work biologists met physicists. Structure of plant genomic DNAs extracted from different tomato cultivars, before and after cryopreservation, were analyzed by surface-enhanced Raman spectroscopy (SERS). Raman spectroscopy is used to observe vibrational, rotational, and other modes in a molecular system. Raman spectroscopy is commonly used in physics and chemistry to provide a fingerprint by which molecules can be identified. However, spontaneous Raman scattering is typically very weak. As compared with normal Raman signals, surface-enhanced Raman scattering cross-sections of molecules residing at or near the surface of roughened or nanostructured materials may be enhanced by factors up to ?10^{14}. Among the studied nucleic acids from leaf tissues, we have identified the DNA, which suffers the weakest structural changes upon cryogenic storage of tomato shoot apices. On the contrary, the most responsive genomic DNA system to cryopreservation process was indicated. However, not significant structural changes of genomic DNAs have been found after cryopreservation in several cases.

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