"All waiting for their time, and time is not waiting for anything" - so claims the old saying. Is it possible that this ancient sentence will lose its validity in the near future? Will it be possible to stop the time and aging process, at least for plants, and how can tiny nanoparticles help in achieving this goal? The answers to these questions will be acquired in the following project.

The protection of biodiversity; i.e. diversity and variability of genes, species, and ecosystems; is one of the most important challenges of the modern world. Over the decades, several strategies for biodiversity protection have been developed. Cryopreservation, i.e. storage of tissues at a cryogenic temperature of liquid nitrogen (-196ºC), is considered the most effective long-term conservation method. However, to make the storage of living biological material at such a low temperature possible, the cells must be properly prepared first. Nanoparticles may be very useful at this point.

Nanoparticles are tiny structures, invisible to the bare eye or even using a classic microscope, with dimensions from 1 to 100 nm (i.e. 10^{-9} m!). Compared with conventional materials, they have higher chemical reactivity and unique physical properties, including increased thermal conductivity. Consequently, the rapid development of nanotechnology has been recently reported. Nanoparticles are widely utilized in medicine, pharmacy, cosmetics industry, and plant protection products. So far, however, they have not been applied in cryopreservation and long-term storage of plant genetic resources. There is still a lack of comprehensive research on the effect of nanoparticles on living organisms.

The following project is focused on the applicability of silver and gold nanoparticles in cryopreservation and their impact on the properties of plants recovered from tissues stored in liquid nitrogen. The penetration of nanoparticles into the cell, their effect on stress reactions, metabolism, and changes in the genetic material, and the appearance of plants will be examined. It is an innovative and pioneering approach, never used before in plant biotechnology.

Two cultivars of bleeding heart will be used as the research material. Due to the ease of cultivation, exceptional beauty, and durability of flowers, as well as decorative leaves, the species is very popular in the horticultural market. Moreover, since the plant is abundant in numerous health-promoting substances, it is also used in the pharmaceutical and cosmetics markets. Despite its great popularity, there are just a few native (endemic) populations of this species left. At the same time, bleeding heart is susceptible to induced mutation occurrence. Consequently, the species is an ideal model organism for research related to biodiversity protection and widely understood plant stability analyses.

During the experiments, silver and gold nanoparticles will be applied, at various concentrations, either during the first (preculture) or second step (encapsulation) of the cryopreservation procedure. By those means, a higher cooling and rewarming pace of the samples is expected. After the storage of tissues in liquid nitrogen and recovery of plants *in vitro*, the effect of nanoparticles on the cryopreservation efficiency will be determined. Imaging of nanoparticles, which are penetrating the cells, is planned using a transmission electron microscope (TEM). This device allows studying the structure of matter at the atomic level (magnification power of several thousand times). The effect of cryopreservation and nanoparticles on the changes in the genome of plants will be investigated using molecular and cytogenetic markers. Biochemical markers; i.e. content of pigments, phenolics, and activity of selected antioxidant enzymes in shoots; will be employed to assess the metabolic stability of cells. Moreover, a detailed evaluation of morphological traits will be performed in plants transferred for further cultivation to the glasshouse.

The results of our experiments will significantly broaden the knowledge about the impact of silver and gold nanoparticles on plants and will be of great importance for both the world of science, especially low-temperature biology and horticultural practice. It will be particularly important to verify the usefulness of nanomaterials in cryopreservation and biodiversity protection. An important aspect of our research is the analysis of possible changes in the genetic material of plants and the level of metabolites synthesis. This information can be used by both producers and breeders of plants, as well as the pharmaceutical and cosmetics industries.

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