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Cryobiotechnology: A Next-Generation Approach to Conserving Plant Diversity

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INTRODUCTION

Climate change coupled with habitat destruction is slowing leading to an erosion of valuable plant species. It has been estimated that nearly three species per year are lost since 1900 due to various natural and anthropogenic factors (Humphreys et al. 2019). This emphasizes the need for conservation of plant diversity to prevent irreversible loss of important genes and traits for future sustainable development. Among the various methods available for biodiversity conservation, scientists are employing the conservation strategy of preserving living cells at -196°C for long term storage. Cryobiotechnology — an intersection of plant science, biotechnology, and ultralow-temperature storage — is emerging as one of the most powerful tools for securing the future of global plant diversity (Pritchard et al. 2017). All metabolic, biochemical and cellular processes stop at the cryogenic temperatures and living cells can be maintained at this temperature for long durations without any change in their genetic constitution.

The science behind the freeze

The main principle governing the success of any cryopreservation procedure is the ability to prevent lethal ice crystallization Three major principles drive this phenomenon: the understanding of water behaviour, prevention of cryoinjury, and use of effective cryoprotectants. Ice crystal formation ruptures cells and cellular membranes leading to cellular damage and death. All cryopreservation procedures aim to avoid intracellular ice nucleation, also termed as seeding, i.e., the point at which ice crystals are initiated, by selecting the most appropriate method of cooling. The cooling rate is critical for cell survival, as both overly rapid and excessively slow cooling can cause damage. During gradual cooling, extracellular ice forms first, creating a water gradient across the cell membrane.

This draws water out of the cells, lowering their internal water content and reducing the risk of intracellular ice formation. If cooling is too slow, however, excessive dehydration concentrates cellular solutes to harmful levels, impairing normal cellular functions. Thus, this approach demands careful optimisation of the cooling rate to remove just enough water to prevent ice formation without inducing colligative injury. Along with this, cryopreservation techniques also exploit the property of water to transition into its amorphous, non-crystalline glassy state, by a process known as vitrification. This can be done by increasing the intracellular solute concentration to increase viscosity, allowing water to vitrify into a glass-like state when exposed to freezing temperatures, inhibiting ice formation.

Cryopreservation techniques

Cryobiotechnology has progressed far from the early slow-cooling methods of the 20th century. Today, researchers use a suite of sophisticated techniques tailored to different plant species.

Technique	Key Features / Description
Controlled-Rate Freezing	Slow, precislely regulated cooling; avoids intracellular ice by gradual dehydration, commonly used for dormant buds
Encapsulation-Dehydration	Explants encapsulated in alginate beads → osmotic dehydration (sucrose) → air/silica drying → LN storage; suitabu for dehydration-sensitive materials
Vitrification	Uses concentrated cryoprotectants (e.g., PVS2) to induce glassy, ice-free state during ultrarapid cooling; widely used for shoot tips
Encapsulation-Vitrification	Combines alginate encapsulation with vitrification solutions; easier handling of delicate explants
Droplet-Vitrification	Explants placed on tiny droplets on aluminiurn foil → extremely rapid cooling and thawing → high recovery rates
V-Cryo Plate Method	Shoot tips fixed on aluminium plates with alginate; dehydration using vitrification solution (PVS2); ultraiar-fast cooling and improved regrowth
D-Cryo Plate Method	Similar plate system, but dehydration is done by air-drying instead of PVS2;

Decoding the Frozen Cell

A major focus of cryobiotechnology is understanding how cells sense, respond to, and survive extreme cold. Maintaining cellular viability at ultra-low temperatures involves a complex interplay of a number of gene expression networks and signalling pathways. Genes involved in cold response, osmotic stress, oxidative stress, and programmed cell death all get activated during the cryopreservation process. Similarly, antioxidant scavenging pathways are activated during the cryogenic storage, removing the generated free radicals and avoiding programmed cell death. Several antioxidant genes, such as copper/zinc superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, catalase and manganese superoxide dismutase, have been found to have increased expression levels in seedlings recovered after cryopreservation.

Building Global Cryobanks

Cryopreservation is well recognised as one of the safest and most reliable methods for long-term conservation of biological diversity. However, very few species have yet been preserved. Further, reproducible protocols for cryopreservation have not been developed in many species. Developing genotype-independent protocols still remains a challenge, especially for species highly sensitive to dehydration or osmotic stress. Future efforts centre on establishing large, coordinated cryobanks capable of preserving the botanical wealth for future development and conserving indigenous plant for generations to come.

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