Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 04, Issue: 01 (JAN-FEB, 2024)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Cryopreservation for *in vitro* Plant Conservation- An Ice Age Innovation

(*H. P. Vadodariya, R. A. Bhimani and R. J. Patel)
Department of Genetics and Plant Breeding, Navsari Agricultural University, Gujarat
*Corresponding Author's email: himanivadodariya@gmail.com

Most commercial and agricultural plants produce orthodox seeds, *i.e.*, seeds that can be dehydrated down to low and safe moisture level without any losses and can easily store at low temperature for extended periods (Roberts, 1973). However, some seeds of plants can not be easily stored, which includes some forest or woody plants with recalcitrant seeds, vegetatively propagated plant species that does not produce seeds (banana) and some plant species which are having highly heterozygous or sterile genotypes (sugarcane, potato). recalcitrant seeds are those seeds that losses viability at low moisture level and cannot be dried to low moisture level to permit their storage at low temperature (Roberts, 1973).

Genetic resources of all these mentioned plants or crop species can be traditionally conserved *ex situ* in field collections, but it has its own limitation in maintenance, safety and efficiency. Indeed, they are more prone to pest, diseases and natural calamities. Traditional methods of conservation often fall short in preserving the viability and genetic integrity of plant species over the long term, especially for recalcitrant species or those with limited seed availability (Matsumoto et al., 2013).

As biodiversity dwindles, the need to conserve plant genetic resources becomes paramount, and cryopreservation stands at the forefront of innovative solutions. Cryopreservation, as a methodology, involves the preservation of plant tissues and cells at ultralow temperatures, often below -150°C, thereby effectively placing them in a state of suspended animation. At this temperature, all cellular division and metabolic functions cease. As a result, the plant material may be preserved indefinitely without being altered and ensuring the long-term storage of genetic information. This technique not only extends the viability of plant species but also maintains their genetic integrity, critical for the preservation of diverse traits essential for sustainable agriculture. (Benson, 2008). As we delve deeper into the intricacies of cryopreservation for in vitro plant conservation, it becomes evident that this technique holds immense promise for addressing the challenges faced in preserving plant genetic resources.

This article aims to unravel the intricacies of cryopreservation techniques, exploring their applications, challenges, and future directions in the in vitro conservation of plant genetic resources.

Cryopreservation Techniques

There are various cryopreservation techniques employed in the in vitro conservation of plant genetic resources, but the core principle of cryopreservation lies in the controlled reduction of temperature to levels where molecular motion nearly ceases, preventing cellular damage. By minimizing ice crystal formation, cryopreservation ensures the viability and genetic integrity of plant tissues or cells over extended periods.

1. **Slow freezing:** Slow freezing is a classical cryopreservation method that involves a gradual reduction in temperature, typically utilizing cryoprotectants to minimize ice crystal formation (Kartha and Engelmann, 1994). The process typically includes the use of cryoprotectants, such as glycerol or dimethyl sulfoxide (DMSO), to protect cells from freezing damage. While effective for some plant species, slow freezing may lead to cellular damage due to ice crystal formation.
2. **Vitrification:** Vitrification is an advanced technique designed to eliminate ice crystal formation by dehydrating plant tissues and rapidly cooling them. Particularly effective for delicate tissues and small explants, vitrification has proven successful in preserving shoot tips, meristems, and embryogenic callus cultures (Sakai and Engelmann, 2007).
3. **Droplet Vitrification:** Droplet vitrification is a modification of the vitrification technique where small droplets of plant tissues are exposed to high concentrations of cryoprotectants before rapid freezing. The small size of the droplets facilitates quick cooling and reduces the risk of ice crystal formation. This method has been particularly effective for the cryopreservation of shoot tips and meristems (Sakai and Engelmann, 2007).
4. **Cryoplates:** Cryoplates are specialized containers designed for the efficient freezing of plant materials. Plant tissues, often in the form of small explants or meristems, are placed on a cryoplate, which is then rapidly cooled. This technique has proven successful for a range of species and is especially valuable for the preservation of genetic resources in genebanks.
5. **Dehydration:** Dehydration is the simplest procedure since it consists of dehydrating explants, then freezing them rapidly by direct immersion in liquid nitrogen. This technique is mainly used with zygotic embryos or embryonic axes extracted from seeds (Engelmann, 1997).
6. **Encapsulation-Dehydration:** This technique involves encapsulating plant embryos or tissues in a matrix, such as alginate beads, followed by dehydration and subsequent cryopreservation. The encapsulation protects the plant material from damage during the freezing process, and dehydration reduces ice crystal formation. This method has been successfully applied to various plant species, including fruit trees and forest trees.
7. **Ultra-Rapid Freezing:** Ultra-rapid freezing methods involve exposing plant tissues to extremely low temperatures within a fraction of a second. This rapid cooling minimizes ice crystal formation, further reducing cellular damage. Techniques such as plunge freezing in liquid nitrogen or direct immersion in liquid nitrogen vapor are examples of ultra-rapid freezing methods.
8. **Pregrowth dehydration:** Pregrowth-dehydration involves pregrowing explants with cryoprotectants, dehydrating them in a laminar airflow cabinet or with silica gel, then quickly freezing them.

Applications of Cryopreservation

1. Conservation of Endangered Species: Cryopreservation provides a lifeline for endangered and rare plant species that are difficult to conserve through traditional methods.
2. Preservation of Clonal Germplasm: Valuable clonal material, such as shoot tips and meristems, can be effectively conserved through cryopreservation.
3. Storage of Orthodox and Recalcitrant Seeds: Both orthodox (e.g., cereals) and recalcitrant (e.g., tropical tree seeds) seeds can be successfully cryopreserved, extending their storage life.
4. Safeguarding Germplasm Collections: Cryopreservation offers a space-efficient and cost-effective means of maintaining large germplasm collections for plant breeding programs.

Major challenges in cryopreservation

1. Cryoinjury and Cell Damage: One of the primary challenges in cryopreservation is the potential for cryoinjury, caused by ice crystal formation and cellular damage during the freezing and thawing processes. The challenge is to minimize ice crystal formation and optimize cryoprotectant concentrations to prevent cellular damage.
2. Optimizing Cryoprotectant Formulations: Developing cryoprotectant formulations that are effective across a wide range of plant species and tissues is a complex task. Different plant materials may require specific cryoprotectant concentrations and combinations.
3. Lack of Standardization: Cryopreservation protocols may lack standardization across different laboratories and institutions and among different species. Standardization is essential for ensuring reproducibility and comparability of results.
4. Post-Thaw Recovery Rates: Achieving high post-thaw recovery rates is essential for the success of cryopreservation. Some plant species and tissues exhibit lower recovery rates, impacting the overall effectiveness of the preservation method. Improving post-thaw recovery rates requires continuous optimization of protocols and understanding the specific requirements of different plant materials.
5. Genetic Stability: Maintaining the genetic stability of plant materials during cryopreservation is crucial. Some plant tissues may undergo genetic changes, mutations, or chromosomal abnormalities during the process.
6. Costs and Infrastructure: Establishing and maintaining cryogenic storage facilities, including liquid nitrogen storage, requires significant financial investment. Additionally, the operational costs of maintaining these facilities, along with skilled personnel, can be substantial.

References

1. Benson, E. E. (2008). Cryopreservation of phytodiversity: a critical appraisal of theory & practice. *Critical reviews in Plant sciences*, *27*(3), 141-219.
2. Matsumoto, T., Sakai, A., & Yamada, K. (1994). Cryopreservation of in vitro-grown apical meristems of wasabi (Wasabia japonica) by vitrification and subsequent high plant regeneration. *Plant Cell Reports*, *13*(8), 442-446.
3. Roberts, H. F. (1973) Predicting the viability of seeds. *Seed Sci. Technol*. 1:499–514.
4. Kartha, K. K., & Engelmann, F. (1994). Cryopreservation and germplasm storage. In *Plant cell and tissue culture* (pp. 195-230). Dordrecht: Springer Netherlands.
5. Sakai, A., & Engelmann, F. (2007). Vitrification, encapsulation-vitrification and droplet-vitrification: a review. *CryoLetters*, *28*(3), 151-172.
6. Engelmann, F. (1997). Importance of desiccation for the cryopreservation of recalcitrant seed and vegetatively propagated species. *Bulletin des Ressources Phytogenetiques (IPGRI/FAO); Noticiario de Recursos Fitogeneticos (IPGRI/FAO)*.