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Cryopreservation and germplasm conservation integrated with plant breeding

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Abstract

Genetic resources from plants contribute to global food security and sustainable agricultural practices; they are the foundation of genetic diversity necessary for crop adaptation and enhancement when faced with changing environmental conditions. Rapid genetic erosion caused by climate change, destruction of habitats, and intensification of agriculture have led to significant losses in the amount of genetic diversity available to breeding programs around the world. Cryopreservation is one of the most reliable ways to conserve plant germplasm over the long term and maintain their genetic stability through ultra-low-temperature storage (-196°C in liquid nitrogen). The purpose of this review is to evaluate comprehensively and critically principles of cryopreservation, methods of cryopreservation, and the integration of cryopreservation with plant breeding systems. Mechanisms of cryo-injury include ice crystal formation, oxidative stress, and destabilization of cellular membranes, which are examined in detail within the course of this article. Various techniques of cryopreservation, such as vitrification, encapsulation-dehydration, and slow freezing, are compared. Additionally, the role of cryoprotective agents, methods for optimizing cryoprotective agents, and toxicity issues are reviewed. Finally, the integration of cryopreservation with modern breeding strategies (marker-assisted selection, genomic selection, genome editing, speed breeding) is critically evaluated and summarized. Recent developments in quick-response omics technologies, nanotechnology, and artificial intelligence have increased the efficiency of using cryopreservation and expanding cryopreservation into new areas. Although progress has been made, existing issues related to genotype dependency, variable rates of regeneration, and infrastructural constraints remain. Advanced breeding coupled with cryopreservation provide innovative methods of optimizing the rate of crop improvement and sustaining agricultural production.

Keywords: Cryopreservation, germplasm conservation, plant breeding, genetic diversity, vitrification, cryoprotectants, genetic fidelity, crop improvement, genomic selection, sustainable agriculture

1. Introduction

Agricultural productivity and food security depend heavily upon plant genetic resources. Plant genetic resources are the various sources of genetic diversity for developing new varieties of crops (Maxted *et al.*, 2012; McCouch *et al.*, 2013) ^[28, 29] such as improved landraces, crops from wild relatives, elite breeding lines, etc. Together, these sources provide the genetic diversity needed for crop improvement. Unfortunately, the extensive use of high-yielding varieties combined with monoculture practices has caused significant damage to agricultural genetic diversity through crop genetic erosion and has caused a reduction in the range of genetic types available for breeding programs (Nagel *et al.* 2024, Roque-Borda *et al.* 2021) ^[31, 40]. The loss of genetic diversity due in part to genetic erosion presents a huge challenge for crop improvement in a changing climate (where there will be continued demands for crop varieties to be produced that exhibit resilience to environmental stresses).

Genetic resources have limitations when using traditional methods like ex-situ storage through gene banks or conservation methods in the natural environment using in-situ conservation. Some species are particularly susceptible to these limitations due to having recalcitrant seed types that are unable to endure drying (Reed *et al.*, 2018) ^[37]. Gene banks (Field Gene Banks) are also subject to hazards from the environment (e.g., weather events), pests and diseases present at the bank, etc. Using cryopreservation has emerged as an alternative to the above for addressing these limitations. Cryopreservation takes advantage of storing plant tissue at low temperature so that all metabolic processes cease, thus preserving the tissue forever with no possibility for

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genetic degeneration (Volk & Walters, 2021) [46, 47].

In recent years more so than in prior years, cryopreservation has transitioned from being an avenue to conserve plant genetic resources (e.g., tissue culture) to being a major component of modern day plant breeding systems. Cryopreservation serves multiple purposes in conjunction with modern advanced plant breeding techniques (e.g., genomic selection & genome editing) whether preserving elite germplasm, enabling hybridization, or assisting in utilizing speed breeding (Varshney *et al.*, 2021) [45]. Due to the impact that global warming, habitat fragmentation, urban sprawl and high-intensity farming have had on plant life over the last few decades, there has been a growing concern across the world for plants' genetic diversity to persist into the foreseeable future. New studies show that many traditional crops and their wild relatives are now near extinction, decreasing the genetic diversity that will be available for future agricultural improvements. The germplasm that is conserved in a gene bank is an important source of genes that can help agriculturalists develop crops that are capable of coping with stresses, producing high quality foods, resisting disease, and adapting to changing environmental conditions. As a result, cryopreservation is now a viable way to provide long-term support for agriculturalists' efforts to achieve global food security, establish climate-resistant agriculture, and help to preserve plant biodiversity. The ability to use cryopreservation along with new genetic and breeding technologies to develop improved varieties of crops faster than before will give agriculturalists an opportunity to meet future worldwide demand for food.

2. Genetic Resources and Their Role in Plant Breeding

Genetic diversity is essential to successful breeding programs because it supplies the variability needed for both selection and adaptation when developing new cultivars. Landraces and wild relatives are particularly important sources of genetic variation, containing traits such as drought tolerance and disease resistance that are not typically found in modern cultivars (Dwivedi *et al.*, 2016) [9]. The incorporation of these traits through introgression has been an important strategy that has aided in improving crops (Dempewolf *et al.*, 2017) [8]. Cryopreservation helps store this genetic material for extended periods, making it possible to use

these resources in future breeding programs. "Cryopreservation is a key part of pre-breeding programs as well as supporting genomics-assisted crop breeding and using genetic resources in today's crop improvement systems (Tyack *et al.*, 2020; Brozynska *et al.*, 2016) [6, 40] by enabling breeders to transfer beneficial traits from landrace and wild relatives into elite cultivars, cryopreservation increases genetic diversity and enhances crop resilience, increasing the sustainability and effectiveness of breeding programs.

3. Principles and Mechanism of Cryopreservation

Cryopreservation preserves cellular viability during long-term storage at ultra-low temperatures; however, cells experience several interconnected stresses collectively termed cryo-injury, including ice crystal formation, osmotic stress, oxidative stress, dehydration, and membrane destabilization. The overall mechanisms and their interactions are illustrated in Fig. 2.

Extracellular ice formation increases solute concentration in the surrounding medium, creating an osmotic gradient that drives water out of the cell, resulting in dehydration and cellular shrinkage. As cooling progresses, membrane lipid phase transitions reduce membrane fluidity and stability, increasing structural vulnerability (Wolfe & Bryant, 2001) [51]. Simultaneously, mitochondrial dysfunction promotes the generation of reactive oxygen species (ROS), causing oxidative damage to lipids, proteins, and nucleic acids. Under rapid cooling or inadequate cryoprotection, intracellular ice formation may occur, leading to severe mechanical damage to cellular structures (John D. Benson, 2019) [4]. During thawing, rapid water influx can cause swelling, membrane rupture, and cell lysis. These interconnected processes collectively determine post-thaw cell survival.

Vitrification is an effective strategy to reduce cryo-injury. It involves ultra-rapid cooling in the presence of high concentrations of cryoprotective agents (CPAs), converting cellular water into a glass-like state without ice crystal formation. By preventing ice formation, vitrification minimizes mechanical, osmotic, and oxidative damage, thereby improving post-thaw viability (Atsushi Sakai & Frank Engelmann, 2007) [41].

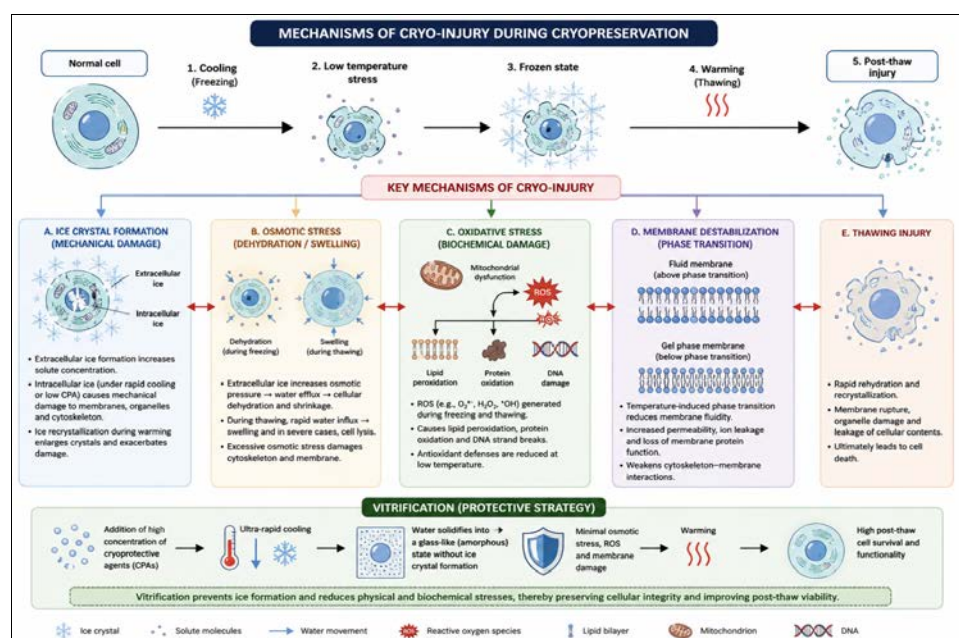


Fig 1: Mechanisms of cryo-injury including ice formation, oxidative stress and vitrification protection

4. Cryopreservation Techniques (Detailed Comparative Analysis)

Several cryopreservation techniques are used for plant and animal biological materials, including vitrification, droplet vitrification, encapsulation-dehydration, and slow freezing. These methods differ in their ability to control ice formation, osmotic stress, and cryoprotectant toxicity, making technique selection dependent on the biological material and experimental objectives.

Vitrification is one of the most widely used techniques because of its simplicity, rapid cooling ability, and high post-thaw survival rates (González-Arno *et al.*, 2014) ^[14]. It involves treatment with highly concentrated cryoprotective agents (CPAs) followed by ultra-rapid cooling, which converts cellular water into a glass-like state without ice crystal formation. Although effective, high CPA concentrations may cause cytotoxicity and osmotic stress if not properly optimized.

Droplet vitrification is a modified form of vitrification in which explants are placed in small droplets of vitrification solution on aluminum foil or cryoplates before immersion in liquid nitrogen. The small sample volume enables faster cooling and warming

rates, reducing ice nucleation and improving survival of sensitive tissues such as shoot tips and embryogenic cultures (Barbara M. Reed *et al.*, 2018) ^[38].

Encapsulation-dehydration involves embedding explants in calcium alginate beads followed by controlled dehydration before freezing (Kantha, 1985) ^[23]. Reduced cellular water content minimizes ice formation, making this method suitable for desiccation-tolerant species, although the process is relatively time-consuming.

Slow freezing, or controlled-rate freezing, uses gradual cooling to allow water to leave the cells before freezing, thereby reducing intracellular ice formation (Niino *et al.*, 1992) ^[32]. This method provides precise control over freezing conditions but requires specialized equipment and may still cause osmotic or ice-related damage if cooling rates are not optimized.

Overall, vitrification and droplet vitrification are generally preferred because of their rapid processing and high survival rates, whereas encapsulation-dehydration and slow freezing are mainly applied in specialized situations depending on species tolerance and available infrastructure.

Fig 1: Comparative overview of cryopreservation techniques

Technique	Advantages	Limitations	Best Use
Vitrification	High survival rate; rapid process; no ice formation	Cryoprotectant toxicity; osmotic stress	Most species and tissues
Droplet vitrification	Ultra-rapid cooling; improved survival; reduced ice formation	Requires technical skill and precision	Sensitive tissues (e.g., shoot tips)
Encapsulation-dehydration	Reduced chemical toxicity; safer handling	Time-consuming; dehydration-sensitive	Desiccation-tolerant plants
Slow freezing	Controlled cooling; reproducible conditions	Expensive equipment; time-intensive	Research and clinical applications

Table 2: Comparative overview of cryopreservation methods used in major crop species and their applications.

Crop Species	Cryopreservation Method	Explant Type	Survival Rate (%)	Regeneration Rate (%)	Major Application	Reference
Banana	Droplet vitrification	Shoot tips	80-95	70-90	Germplasm conservation	Panis <i>et al.</i> , 2021 ^[33]
Potato	Vitrification	Shoot apices	75-90	65-85	Preservation of elite lines	Volk & Walters, 2021 ^[46, 47]
Cassava	Encapsulation-dehydration	Somatic embryos	60-80	50-75	Vegetative germplasm conservation	Reed <i>et al.</i> , 2018 ^[37]
Rice	Slow freezing	Embryogenic callus	65-85	55-80	Breeding studies	Wang <i>et al.</i> , 2017 ^[49]
Sugarcane	Vitrification	Meristem cultures	70-88	60-82	Disease-free germplasm storage	Ballesteros <i>et al.</i> , 2024 ^[2]
Medicinal plants	Encapsulation-vitrification	Shoot cultures	55-78	45-70	Biodiversity conservation	Kaviani, 2021 ^[24]

5. Cryoprotectants and Optimization

Cryoprotectants are essential for minimizing ice crystal formation, maintaining osmotic balance, and protecting cellular structures during freezing and thawing. They are broadly classified into penetrating and non-penetrating cryoprotectants (Barry J. Fuller *et al.*, 2004) ^[11, 12].

Penetrating cryoprotectants, such as dimethyl sulfoxide (DMSO), glycerol, and ethylene glycol, diffuse into cells and prevent intracellular ice formation by lowering the freezing point and stabilizing proteins and membranes. DMSO is widely used because of its high permeability, although high concentrations may cause cytotoxicity.

Non-penetrating cryoprotectants, including sucrose and trehalose, mainly act extracellularly by promoting osmotic dehydration and reducing intracellular ice formation. Trehalose

also stabilizes membranes and proteins by forming a protective glass-like matrix during dehydration (Arakawa *et al.*, 1990) ^[57].

A major limitation of cryopreservation is cryoprotectant toxicity, particularly at high concentrations required for vitrification. Therefore, optimization of concentration, exposure time, and temperature is critical for effective preservation.

Recent advances include the use of antioxidants such as ascorbic acid, glutathione, and melatonin to reduce oxidative stress, as well as nanotechnology-based approaches to improve cryoprotectant delivery and reduce toxicity (Kang *et al.*, 2025) ^[22].

Overall, combining penetrating and non-penetrating cryoprotectants with improved preservation strategies enhances post-thaw survival and cellular functionality.

Table 3: Cryoprotectants and Their Functions

Cryoprotectant	Type	Mechanism of Action	Advantage	Limitation
DMSO	Penetrating	Prevents intracellular ice formation	High efficiency	Toxic at high concentration
Glycerol	Penetrating	Stabilizes proteins and membranes	Low toxicity	Slow penetration
Ethylene glycol	Penetrating	Enhances vitrification	Fast penetration	Moderate toxicity
Sucrose	Non-penetrating	Promotes dehydration	Safe	Limited intracellular protection
Trehalose	Non-penetrating	Protects membranes	Stabilizes cells	Expensive

6. Regeneration and Plant Recovery

Successful regeneration after cryopreservation is considered the ultimate indicator of viability and functional recovery of preserved plant material, since post-thaw survival alone does not ensure normal growth and development. Regeneration efficiency is influenced by several factors, including genotype, explant type, cryoprotectant treatment, and post-thaw culture conditions (Wang *et al.*, 2017; Frank Engelmann, 2011) [10, 49]. Following thawing, cells must restore membrane integrity, repair oxidative damage, and reactivate metabolic and developmental pathways. The efficiency of these recovery processes largely determines successful regeneration (Barry J. Fuller *et al.*, 2004) [11, 12]. Genotype plays a major role in regeneration potential, as different species and cultivars vary in their tolerance to cryo-

induced stress due to differences in membrane composition, antioxidant systems, and stress-response mechanisms (Atsushi Sakai & Frank Engelmann, 2007) [41]. Explant type is also important, with meristematic tissues such as shoot tips and embryogenic cultures generally showing higher regeneration ability because of their active cell division and developmental plasticity. Cryoprotectant concentration and exposure time must be carefully optimized to balance cryoprotection and cytotoxicity. Excessive exposure may impair cellular recovery and reduce regeneration efficiency (John D. Benson, 2019) [4]. In addition, post-thaw culture media and plant growth regulators (PGRs), especially auxins and cytokinins, are essential for supporting cell division, differentiation, and organogenesis (Joseph Street, 1977) [42].

Table 4: Factors affecting regeneration after cryopreservation:

Factor	Effect on Regeneration
Genotype	Determines survival variability
Explant type	Influences regeneration capacity
Cryoprotectant concentration	Affects toxicity and viability
Culture media	Controls growth and recovery
Growth regulators	Enhance regeneration

Overall, successful regeneration depends on the careful optimization of both pre- and post-cryopreservation conditions to improve recovery and ensure the effective use of cryopreservation in plant conservation and biotechnology.

7. Evaluation of Agronomic Traits

To verify the effectiveness of the cryopreservation process, it is critical to evaluate the agronomic characteristics of the regenerated plants; in addition to surviving post-thaw, the regenerated plants must grow normally, produce normally, and be genetically stable while growing in the field. Normal parameters are measured after thawing, including height, biomass, yield, flowering characteristics, and tolerance to abiotic stress. Comparative evaluations (between the cryopreserved and non-cryopreserved controls planted in parallel) allow researchers to assess the extent to which cryo-induced stress resulted in a difference among the cryopreserved plants when compared with non-cryopreserved controls. The majority of comparative studies show that when compared with non-cryopreserved plants, the morphological, physiological, and agronomic characteristics of cryopreserved plants have not been significantly altered by the cryopreservation process. For example, potatoes and bananas both exhibit normal growth characteristics and agronomic performance following cryopreservation (Panis *et al.*, 2021) [33]. In addition to assessing observable characteristics of regenerated plants (phenotypes), investigators also utilize molecular markers and cytological analysis to assess the fidelity of the regenerated

plants' genetic information to assure that somaclonal variation and genetic change did not occur as a result of the cryopreservation processes. Genetic stability is particularly important in the preservation of germplasm and in plant/crop improvement programs. The overall performance of regenerated, cryopreserved plants in field settings demonstrates that cryopreservation is a viable option for long-term conservation of plant germplasm, given that the appropriate procedures are followed.

8. Genetic Fidelity and Molecular Tools

The conservation of genetic fidelity during cryopreservation is critical for the use of conserved germplasm in plant breeding programs. While volatilization may be an effective means of halting cellular metabolism and is generally regarded as being genetically stable, dehydration, cryoprotectant exposure, freezing, and thawing all result in stress that may introduce small genetic or epigenetic variations that can be detected by molecular markers or via comparison to non-cryopreserved samples. As such, genetic stability testing is very necessary to confirm whether or not the genetic integrity of a cryopreserved sample is maintained (Harding, 1994) [16]. There are several molecular marker systems available for assessing genetic fidelity; among the most commonly used are simple sequences repeat (SSR), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphisms (SNP) markers. SSR are particularly valuable as they are highly polymorphic and reproducible and the SNP have also allowed for genome-wide analysis at a high resolution (Varshney *et al.*

2021) [45]. In a number of comparative studies, cryopreservation-derived plants have demonstrated little to no genetic variation when compared to their non-cryopreserved controls (Ibáñez *et al.*, 1999; Peredo *et al.*, 2008) [18, 36] thus suggesting that optimized cryopreservation processes can keep genetic fidelity intact.

In addition to DNA analyses, epigenetic changes such as changes in methylation of DNA have been reported. These changes may also regulate gene expression; thus, in addition to regulating the expression of certain genes, epigenetic changes could also have an effect on the phenotype of the plant. Methylation-sensitive amplified polymorphisms (MSAP)

and bisulfite sequencing have been used to detect epigenetic changes in cryopreserved materials. Although there are several techniques used to analyze complex traits (e.g., whether a plant expresses a specific trait; i.e., size and/or shape) or to evaluate genetic fidelity in plants, the most reliable method is still unclear and more work is needed in this regard. Furthermore, continuing to evaluate cryopreserved material and its epigenetic changes relative to non-cryopreserved controls should occur so that appropriate cryopreservation protocols can be developed to preserve genetic fidelity and allow for subsequent use in breeding programs.

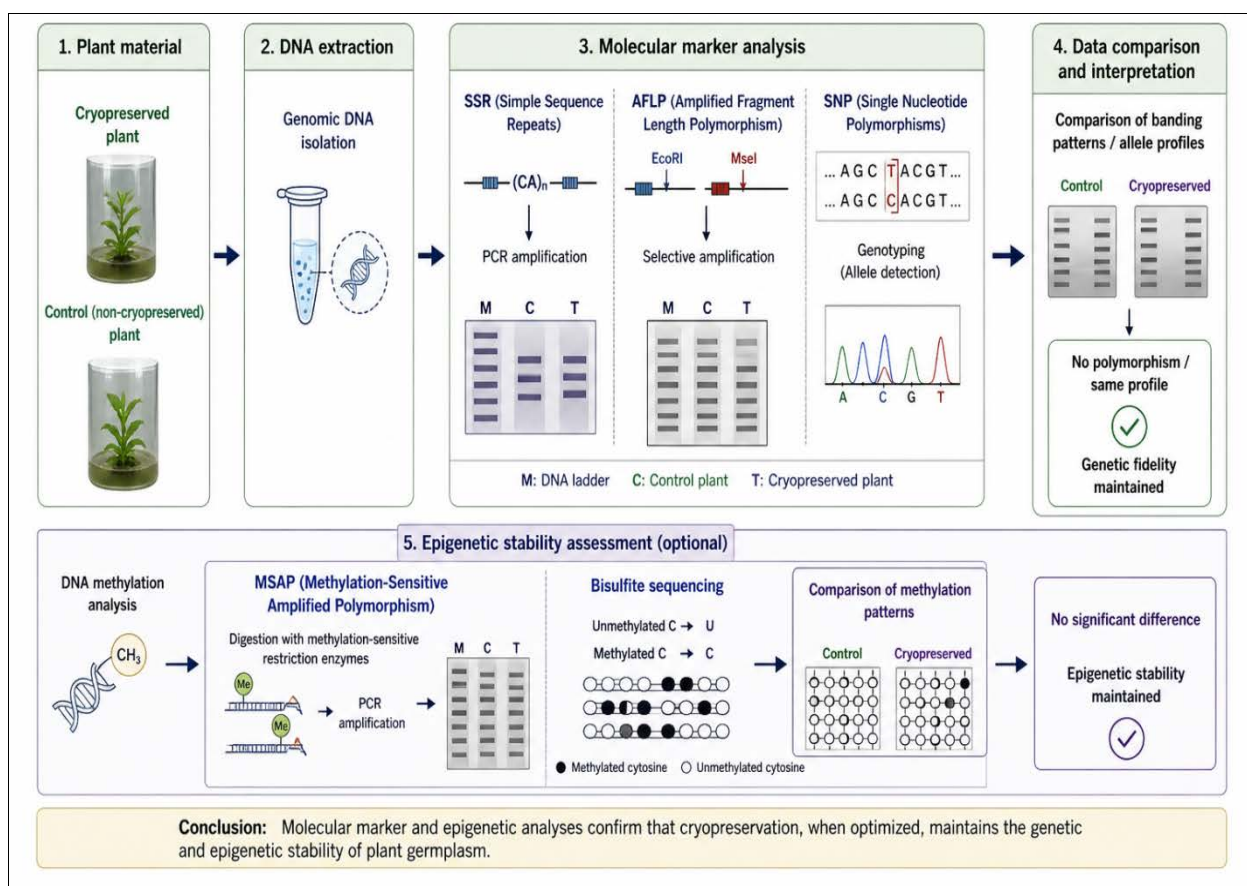


Fig 2: Use of molecular markers (SSR and SNP) for assessing genetic fidelity of cryopreserved germplasm

9. Integration with Plant Breeding Strategies (Critical Analysis)

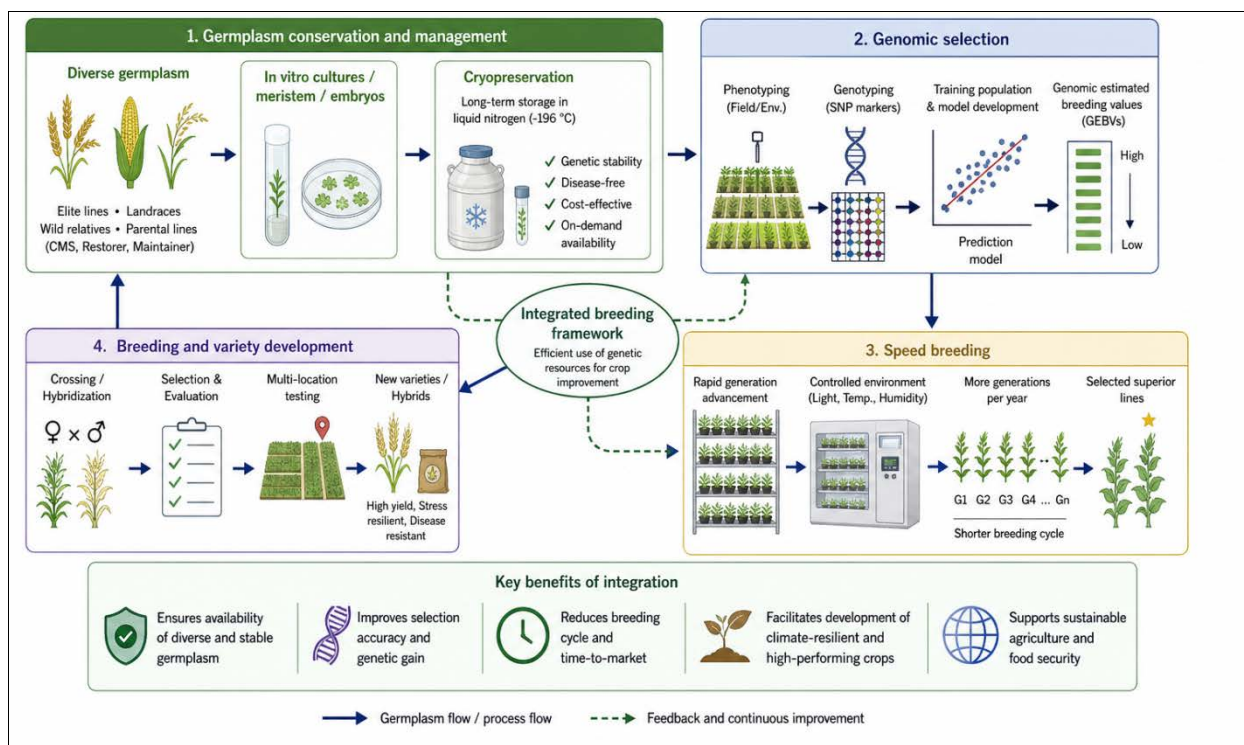
Cryopreservation has become an integral part of modern plant breeding systems, facilitating long-term storage and effective utilization of genetic resources. Integration with breeding programs enhances genetic gain by reducing breeding cycles and providing different germplasm for crop improvement (Crossa *et al.*, 2017) [7].

In hybrid breeding programs, cryopreservation is particularly useful for preserving parental lines such as Cytoplasmic male sterile (CMS) lines and restorer lines. These lines are essential for hybrid seed production, and their preservation ensures uniformity and stability in breeding programs. Cryopreservation removes risks in field maintenance such as drift and disease.

Cryopreservation is crucial in pre-breeding programs since those

programs preserve wild relatives and landraces that are the sources of everything from novel traits. Such genetic resources may be applied to a wide range of traits to be replaced to elite cultivars concerning drought tolerance, salinity resistance, and disease resistance values. Our role in this context is becoming highly sensitive under climate change circumstances.

The combination of cryopreservation with advanced breeding technologies such as genomic selection and speed breeding has hugely enhanced breeding efficiency. Genomic selection uses genome-wide marker information to predict breeding values, while speed breeding enhances turnover of generations and accelerates the crop improvement programs (Watson *et al.*, 2018) [50]. At this point in time, cryopreservation provides backup to these approaches by storing germplasm.



Source. Made using chatgpt

Fig 3: Integrated breeding framework combining cryopreservation, genomic selection, and speed breeding

9A. Cryopreservation in Indian Germplasm Conservation Programs

India is a key producer of various types of crops, including but not limited to: cereals, pulses, oilseeds, fruits, vegetables, medicinal plants and their wild relatives. Many of these crops have genetic resources that can be conserved through the efforts made by the National Bureau of Plant Genetic Resources, which is part of the Indian Council of Agricultural Research (ICAR). The National Gene Bank collects, stores and preserves genotypes from seeds, both those officially registered as traditional and those not (Malik *et al.*, 2010; Pandey & Tyagi, 2018) [27, 34].

Below zero temperatures (cryopreservation) provide a means of conserving non-storables (crops that cannot be combined into traditional methods of long-term seed storage, including monocotyledons such as bananas, potatoes, cassavas and sweet potatoes). Several techniques are used during the cryopreservation process, including shoot-tip cryopreservation, preservation of embryonic cultures, and storage of pollen (Reed, 2018; Volk & Walters, 2021) [46, 47]. For example, banana and potato cryopreservation has allowed large-scale long-term storage of healthy, elite genetic materials while maintaining genetic integrity of the genetic materials (Panis *et al.*, 2021; Volk & Walters, 2021) [46, 47, 33]. Cryopreservation efforts are also currently underway for the purposes of keeping intact the genetic stocks of rare flowers (medicinal plants) and providing genetic material for orchids (Engelmann, 2011; Kaviani, 2021) [10, 24].

In addition to preserving genetic stocks, cryopreservation assists in continuing crop improvement by providing the base for continued crop improvements by providing access to elite crop stock with commercial potential or wild relatives with desirable traits i.e. drought tolerance / salinity resistance / disease resistance as well as increasing the ability to use proprietary genetic materials through molecular characterization and genomics-assisted breeding methods.

B. Applications in Crop Improvement

The function of cryopreservation is to store and conserve valuable plant genetic resources through a method of long-term storage for the purpose of plant improvement. This is particularly true for plants that are propagated vegetatively (i.e., potatoes, bananas, cassavas, and sweet potatoes) (Panis *et al.*, 2021) [33]. Cryopreservation provides a way to maintain elite germplasm and parental lines and protects the germplasm from diseases so that it may be used for genetic breeding and research.

Long-term storage using cryopreservation has been consistently documented with successful regeneration of potatoes and bananas with no loss of agronomic performance, thus benefitting breeding programs focused on yield, quality, and resistance to disease (Volk & Walters, 2021) [46, 47]. Furthermore, cryopreserved storage of wild relatives has preserved important traits that could be utilized in more successfully developing climate-resilient crops, including some that give plants an ability to withstand drought and disease. Lastly, cryopreservation provides support for plant biotechnology by preserving transgenic lines, somatic embryos and *in vitro* cultures and by minimizing risk of genetic instability associated with the long-term maintenance of the plants. This also has strengthened the efficiency of breeding through the conservation of genetic diversity and by ensuring that genetically stable and high quality germplasm are available for future crop improvement activities.

10. Physiological and Biochemical Responses

Cryopreservation causes a number of physiological and biochemical reactions in plant tissue through dehydration, osmotic pressure, and freezing damage. One of the main responses is the formation of reactive oxygen species (ROS), which can create oxidative damage to cellular structures including lipids, proteins, and nucleic acids (Apel & Hirt 2004) [1].

Plants have antioxidant defence mechanisms that reduce

oxidative stress. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), quench ROS by converting them to less damaging substances (Gill & Tuteja 2010) ^[13]. Increased activity of these enzymes in cryopreserved tissue suggests that they may assist in survival following cryopreservation.

Membrane stability is also an important factor in the success of cryopreservation. Freezing stress can alter the membrane structure, which can cause the membrane to become more permeable and leak the contents of the cell. Electrolyte leakage tests have been used as a method for assessing the integrity of membranes.

11. Challenges and Limitations

The limitations of cryopreservation notwithstanding its many advantages are several, which create barriers to implementing it on a large scale. One of the main challenges is genotype dependence; many cultures fail to regenerate or develop into mature plants due to their individual responses to freezing, dehydration and recovery conditions (Benson, 2008; Reed, 2008) ^[3, 39]. Therefore, universal protocols will be difficult to establish and will require optimization on a species by species basis.

A further limitation applies when plants exhibit low regeneration rates after exposure to cryogenic temperatures and tissues may survive cryogenic conditions but are unlikely to be able to produce healthy plants from that tissue because of higher rates of injury and death (Engelmann, 2011; Volk and Walters, 2021) ^[46, 47]. Another concern relative to cryopreservation is that cryopreservation agents may be toxic. Cryoprotectant agents can cause osmotic stress, membrane damage and metabolic disruption, for example, DMSO, glycerol and vitrification solutions such as PVS2 (Sakai and Engelmann, 2007; Kim, *et al.*, 2009) ^[26, 41]. Therefore, the challenge is to identify the optimal conditions for cryopreservation without subjecting the cells or tissues being preserved to significant toxicity.

Specialized infrastructure is required to carry out cryopreservation procedures effectively; a supply of liquid nitrogen, controlled-rate freezers, sterile tissue culture facilities and trained personnel are all essential to successful application. The high costs associated with the acquisition of these items will limit the implementation of cryopreservation technologies in many developing countries (Kaviani, 2011; Walters *et al.*, 2013) ^[25, 48]. The above limitations have made it difficult to cryopreserve many plants on a large scale.

12. Recent Advances (2020-2025)

Recent advances in cryopreservation have significantly improved its efficiency and applicability. Innovations in vitrification techniques, including droplet vitrification and encapsulation-vitrification, have enhanced survival rates (Ballesteros *et al.*, 2024) ^[2].

The integration of cryopreservation with omics technologies such as genomics, transcriptomics, and metabolomics has provided new insights into cellular responses during cryopreservation. Nanotechnology and artificial intelligence are emerging as promising tools for optimizing protocols.

13. Future Perspectives

Future advancements in cryopreservation will be primarily based on combining multiple different scientific fields; biomolecular treatments or biotechnology, intelligent systems developed with Artificial Intelligence; the delivery of precise dosage using tiny machines (nanotechnology); and knowledge gained from

genome science. In addition, the use of machine learning algorithms has made it possible for researchers to create intelligent cryopreservation systems that can predict optimal dehydration levels, combinations of cryoprotectants, and rates of cooling, leading to an improvement in the percentages of success in surviving and regenerating.

Artificial intelligence-based predictive models will be developed to assist researchers in determining genotype-specific responses to the stresses of cryogenic preservation; therefore, reducing the amount of trial-and-error that researchers may need to conduct. The advantages of using nanotechnology will include but are not limited to improved cryoprotectant delivery systems through reduced toxicity levels and enhanced thermal properties during both the freezing and thawing process.

The potential of cryopreservation in support of new crop development through the use of genetic engineering (CRISPR/Cas) and genomic selection technologies to support modern crop seed breeding programs will continue to grow. Cryopreservation will provide an opportunity for genetic improvement using edited germplasm and elite seed lots to develop fast-track animal and crop breeding programs. Many developing countries will require further research focused on the thermal control of cryogenic preservation. Automation of liquid nitrogen utilities, digital monitoring systems, and their integration into cryogenic robotic storage systems will increase cryogenic operational efficiencies and reduce maintenance costs. Also, integrating various forms of omics technologies (genomics, transcriptomics, proteomics and metabolomics) to study the cellular responses of freezing and thawing will also continue to develop. Such approaches may contribute to discovering ways to reduce cellular damage during freezing and thawing.

14. Conclusion

Long-term conservation of plant genetic resources will be possible using cryopreservation because it is a reliable and effective means of ensuring the conservation of biodiversity and the long-term improvement of crop species. The biological processes of the plant are essentially halted at ultra-low temperatures, providing an effective method of preserving valuable germplasm from a variety of sources (e.g., vegetative crops propagated through asexual reproduction, elite breeding lines, and genetic materials in danger of extinction) without affecting their genetic integrity.

Technological improvements in cryopreservation techniques, a better understanding of cryo-injuries, as well as the continued development and optimization of cryoprotectants, have greatly increased the survival of post-thaw plant germplasm and the efficiency with which they are regenerated. The use of modern biotechnology in conjunction with cryopreservation techniques (e.g., CRISPR/Cas system and molecular breeding techniques) creates an even greater potential for developing high-yielding, stress/ drought-resistant, and disease-resistant crop varieties than with cryopreservation alone. Despite a range of obstacles (e.g., genotype-specificity, cryoprotectant toxicities, and infrastructure limitations) to cryopreservation research and adoption of new techniques, continued technological and research innovations are anticipated to resolve these barriers. Emerging methods of enhancing the efficiency and accessibility of cryopreservation techniques, such as those incorporating nanotechnology, artificial intelligence, and increased standardization of protocols, may contribute to achieving these objectives.

Overall, cryopreservation will serve as a core technology for the development of a sustainable agricultural system through the

conservation and utilization of plant genetic diversity in order to meet global challenges of food security, climate change, and environmental sustainability.

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