Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 06, Issue: 01 (JAN-FEB, 2026)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Cryopreservation of Seed

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Cryopreservation is a process that protects biological matter, such as cells, tissues, or even entire organs, from decay by storing them at extremely low temperatures, typically in liquid nitrogen at -196°C. This deep freeze stops all metabolic activity and biological processes, preventing cellular damage and preserving the sample's viability for future use. The process requires the use of cryoprotective agents (CPAs) to prevent cell damage from ice crystal formation during the freezing and thawing cycles. Viable freezing of biological material and their subsequent storage at ultra low temperatures (-150 to -196° C) in liquid nitrogen . Biological, biochemical and physiological activities stopped due to less temperature then, plant materials could be stored for unlimited years Represents safe and cost-effective option for long-term conservation of germplasm.

Types of Tissue Covered Under Cryopreservation

- Seeds and pollen
- Zygotic embryos
- Embryonic cell suspensions
- Somatic embryos
- Meristem/shoot tip cultures etc.

Material used

- Liquid nitrogen is most widely used material for cryopreservation
- Dry ice (Solid carbon dioxide) can also be used

Methods of cryopreservation

1. Conventional method

- Addition of an appropriate cryo protectant
- Subjection of culture to super low temperatures
- Storage of frozen culture in liquid nitrogen
- Thawing
- Removal of cryoprotectant by washing
- Viability Determination
- Reculture and Induction of growth and plant regeneration

2. Recently Developed or Modified Methods

A. Vitrification

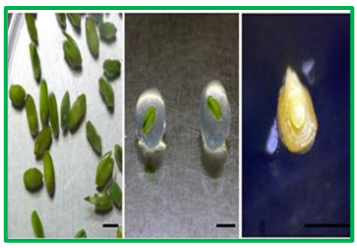
It ia a Physical process of transition of an aqueous solution into an amorphous and glassy state or the Process in which ice formation cannot take place because aqueous solution is too concentrated to permit ice crystals nucleation. Instead, water solidifies into an amorphous 'glassy' state. Avoids most damaging event ie. formation of intercellular ice crystals during

cryopreservation. In this process Cells are dehydrated by treatment in a highly concentrated solution such as PVS (Plant Vitrification Solution) solution.

B. Encapsulation-dehydration

This method was first reported by Fabre and Dereuddre (1990) using shoot apices of potato.This involves encapsulation of tissues in Calcium/Sodium alginate beads which are pre-grown in liquid culture media containing high concentration of sucrose.After these treatments tissues are able to withstand exposure to liquid nitrogen without application of chemical cryoprotectants.

C. Droplet vitrification



Technique- modification of basic vitrification protocol. It involves placing the sample within a droplet of 1-10 µl cryoprotective solution on a piece of aluminium foil before immersion in liquid nitrogen. This approach achieves higher cooling and re-warming rates, as small volume of liquid allows higher rate of heat transfer to and from sample (Sakai & Engelmann, 2007).

Cryopreservation steps

1. Plant material selection

Explant's morphological and physiological condition influences ability to survive during cryopreservation

Considerable factors

- Tissue selection- from healthy plants
- Small, young, rich in cytoplasm and meristematic cells- Can survive better than larger and highly vacuolated cells
- Callus-freezing damage resistant
- Cell or tissue should contain low water content for cryopreservation then only tissues withstand extreme low temperatures.

2. Pre-growth

It protects plant tissues against exposure to liquid nitrogen and involves application of additives (Abscisic acid, Proline, Trehalose etc.)to enhance plant stress tolerance. In this partial tissue dehydration achieved by application of osmotically active.

3. Freezing

A. Rapid/Fast freezing

- Employed for shoot tip cryopreservation-Potato, Strawberry. Brassica species etc.
- Material placed in vials/tubes and plunged into liquid nitrogen
- Temperature reduction from-300 to-1000°C/min or more occurs
- Prevents growing of big ice crystals

B. Slow freezing:

- Large ice crystals
- Slower freezing rate
- More time needed for the freezing process

4. Addition of cryoprotectants

Cryoprotectant- Substance used to protect biological tissue from freezing

- Acts like antifreeze
- Lowers freezing temperature
- Increases viscosity
- Prevents cell damage

Various cryoprotectants used are: Glycerol, Dimethyl Sulphoxide (DMSO), Sugar, Mannitol, Propylene Glycol (PEG) etc.

5. Thawing

It is done by putting ampoule/tube containing frozen tips of sample in warm water bath (35) to 40°c) with vigorous swirling action.

6. Storage

Storage of frozen material at correct temperature is as important as freezing

- Frozen cells/tissues kept for storage at temperature ranging from -70 to-196°c
- Low temperature for longer period-To stop all metabolic activities and prevent biochemical injury
- Best done at-196°C

Conclusion

Cryopreservation is an effective and reliable technique for the long-term conservation of plant germplasm by storing biological materials at ultra-low temperatures, usually in liquid nitrogen. At such temperatures, all metabolic and physiological activities are completely halted, preventing cellular deterioration and maintaining genetic stability for extended periods. The use of cryoprotective agents and advanced methods such as vitrification, encapsulation–dehydration, and droplet vitrification helps minimize freezing injuries, especially damage caused by ice crystal formation. Overall, cryopreservation represents a safe, cost-effective, and efficient strategy for preserving valuable plant genetic resources and supporting future research, breeding, and conservation programs.

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