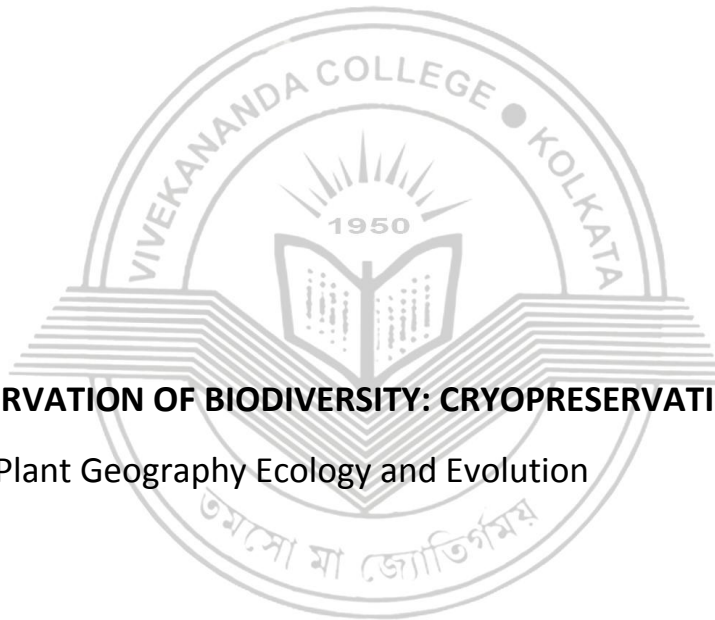


VIVEKANANDA COLLEGE

THAKURPUKUR

KOLKATA-700063

NAAC ACCREDITED 'A' GRADE



Topic: **CONSERVATION OF BIODIVERSITY: CRYOPRESERVATION**

Course Title: Plant Geography Ecology and Evolution

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Cryopreservation: An *ex situ* method of conservation of plant germplasm

Cryopreservation

The term 'cryopreservation' (cryogenic preservation) refers to the storage biological materials like cells, tissues and organs at the ultra-low temperature of liquid nitrogen (-196°C). At this temperature, the vegetative cells enter in a state of "absolute quiescence", as all the physical and biochemical reactions are practically halted; in this particular condition, conservation time becomes unlimited. . When the cells are led to this extreme ultra-cold condition following appropriate "preparatory" procedures, their viability is preserved and, when brought back to standard culture conditions, they can recover full functionality.

Application of cryogenics to the conservation of plant material, proposed for the first time in the year 1968 for the maintenance of cell cultures, is today a reality for the conservation of differentiated organs and tissues as well. Now, this is an effective *ex situ* method for conserving germplasm of rare and endangered plants.

The main **advantages** of cryopreservation are:

- Cryopreservation is the only method currently available to ensure the safe and cost-effective long-term conservation of genetic resources of species that have recalcitrant seeds or are vegetatively propagated.
- A wide range of plant organs and tissues, shoot tips from in vitro culture, embryonic axes, somatic embryos, seeds, bulbils, buds, pollens can be put in conservation.
- Large quantities of materials can be preserved in small space.
- The germplasm preserved can be maintained in an environment, free from pathogens.
- Plant materials from endangered species can be conserved and protected against the nature's hazards.
- From the germplasm stock, large number of plants can be obtained whenever needed.
- Furthermore, in liquid nitrogen it is possible to preserve cell and hairy root cultures producing secondary metabolites of industrial and pharmacological interest, as well as callus cultures, embryogenic and/or genetically transformed.
- Cryopreservation is a good method for the selection of cold resistant mutant cell lines which could develop into frost resistant plants.
- Recently the cryogenic technology is used to the conservation of germplasm woody plant like fruit trees, timber yielding and ornamental species.

The main **disadvantages** of cryopreservation are:

- Formation ice crystals inside the cells should be prevented as they cause injury to the organelles and the cell.
- High intracellular concentration of solutes may also damage cells.
- Sometimes, certain solutes from the cell may leak out during freezing.
- Cryoprotectants also affect the viability of cells.
- The physiological status of the plant material is also important.
- One major limitation of germplasm storage is the expensive equipment and the trained personnel.

Protocol of Cryopreservation

1. Development of sterile tissue cultures: Any tissue from a plant can be used for cryopreservation e.g. meristems, embryos, endosperms, ovules, seeds, cultured plant cells, protoplasts, calluses are grown on growth medium.

2. Gradual mixing of Cryoprotectant solution with plant material and pretreatment: Cryoprotectants are used to prevent the damage caused to cells by freezing or thawing. As a result, the ice crystal formation is retarded during the process of cryopreservation.

3. Freezing: It is done either by slow cooling (cooling rate 0.5-4⁰C per minute from 0⁰C to -100⁰C and then to liquid nitrogen) or by rapid cooling or initially slow and then rapid. A partial dehydration of the material before freezing help to increase the survival of cells/tissues. For this purpose Plant Vitrification solution (PVS) like ethylene glycol, sucrose etc. is used.

The dehydrated tissues/cells are then frozen by directly immersing them in liquid nitrogen. The cytoplasm of the PVS-treated cells freeze in a non-crystalline glass like structure. This process is called vitrification.

4. Storage: The plant materials are kept in 1-2ml ampoules of polypropylene along with cryoprotectant solution dissolved in culture medium. Frozen tissues are stored in liquid nitrogen refrigerator at -196⁰C.

5. Thawing: Vials are plunged into water at 35⁰-40⁰C for 90 seconds, then transferred to water bath at 25-30C temperature. The materials are then transferred to ice-bath till reculture.

6. Re-culture: Cryoprotectant solution is removed by washing with water and cells are directly placed on culture medium.

7. Measurement of survival/viability

8. Plant regeneration: To maintain maximum genetic stability, callus formation is to be avoided.

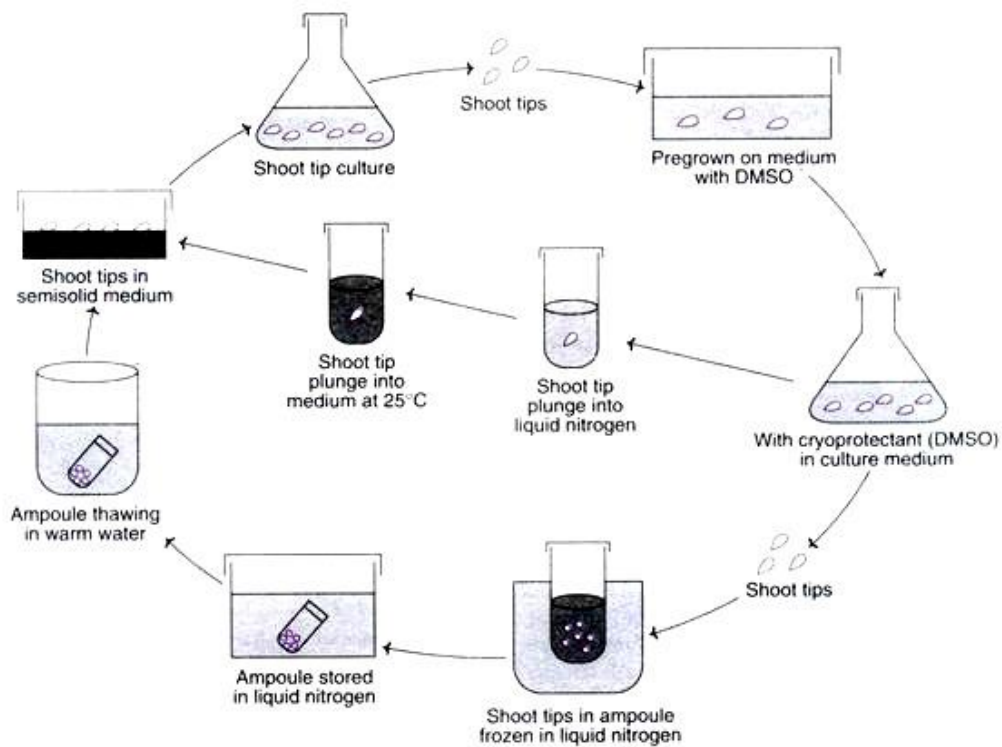


Figure: Outline of the Protocol of Cryopreservation of Shoot Tip

Glossary

Germplasm: Germplasm is a live information source for all the genes present in the respective plant, which can be conserved for long periods and regenerated whenever it is required in the future. Germplasm of a crop plant can be defined as the sum total of hereditary material i.e. all the alleles of various genes present in a crop and its wild cultivars. Germplasm conservation is the most successful method to conserve the genetic traits of endangered and commercially valuable species

Cryoprotectants: Cryoprotectants are the compounds that can prevent the damage caused to cells by freezing or thawing. The freezing point and super-cooling point of water are reduced by the presence of cryoprotectants. As a result, the ice crystal formation is retarded during the process of cryopreservation. Example-dimethyl sulfoxide (DMSO),glycerol, ethylene, propylene, sucrose, mannose, glucose, proline and acetamide; DMSO, sucrose and glycerol are most widely used.

Assignments

Type-A: Answer the following questions: [2 marks each]

1. What is cryopreservation?
2. Define the term 'Germplasm'.
3. Mention the importance of cryoprotectants.
4. What is PVS? Name one.

Type-B: Answer the following questions: [5 marks each]

1. Write a short note on the role of cryopreservation in conservation of plant germplasm.

