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Cryopreservation: A Scientific Approach

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Biomaterials are preserved at very low temperatures throughout the cryopreservation procedure. This is often carried out at –196°C (–321°A) in liquid nitrogen, or sometimes at –80°C (–112°F) in solid carbon dioxide (dry ice). As long as biological materials have stopped their activities and metabolism at cryogenic temperatures (below –100°C), they may theoretically be thawed to normal physiological temperatures and then somewhat reanimated. Actually, a dip in body temperature below freezing, or "cryo-hibernation," occurs naturally in a number of amphibian species. These animals have developed defence mechanisms to help them withstand the harsh winters. For instance, in order to prevent the development of ice, wood frogs (Rana sylvatica) can raise the quantities of urea and glucose in their bodily fluids. Cryopreservation technology is considered to be a tool for preservation of biological materials in perpetuity and has been applied in many fields.

Cryopreservation

Biological materials are preserved or conserved with the use of cryogenic temperatures, which are typically below 180°C. Germplasm is the term for the genetic materials of germ cells, such as seed, sperm, or eggs, that have been cryopreserved. preservation by freezing. Vitrification is another name for the cryopreservation of biological materials at a very fast cooling pace without the creation of ice crystals.

It is vital to preserve plant genetic resources in whatever way possible in order to stop the decline in plant biodiversity. There are two main approaches to conservation: in situ and ex situ. In situ conservation is the preservation of germplasm in its native environment. The preservation of germplasm in gene banks is referred to as ex situ conservation. The complete genetic variety of a crop species can be preserved in one location with relatively less expense and less extensive management thanks to ex situ conservation strategies. Seed gene banks, field gene banks, botanic gardens, in vitro banks, cryogene banks,

and DNA banks are examples of ex situ conservation techniques. When creating long-term base collections of crops for use in the far future, liquid nitrogen (LN) storage is typically used. LN serves as a safety precaution for similar collections kept in clonal repositories, field gene banks (FGB), or in situ conserved genetic material, which is vulnerable to natural whims. (Anushma 2020).

Principles of Cryopreservation Technology

The materials being cooled typically consist of biological cells, buffers that are intracellular (found inside the cell), and cryoprotectants that are dissolved in buffer that is extracellular (found outside the cell). Quantifying the biological, chemical, and physical changes that occur during the cooling and warming (thawing) processes is one of the main challenges in cryopreservation technology. These studies specifically address the following topics: changes in cell size and structure; additional or extracellular ice formation and heat transfer; effects of cooling and warming rates; and the exchange of water, cryoprotectant, and ions across the cell membrane (a semi-permeable membrane that permits only certain substances to pass through while keeping others in or out). Many single cells, including sperm, and blood cells, including erythrocytes, lymphocytes, and hemopoietic cells, were studied experimentally. Important discoveries were made into the mechanisms underlying cell damage during cryopreservation.

Procedure of Germplasm Cryopreservation

1. Quality control and sample collecting
2. Cryoprotectant addition and equilibration
3. Sample labelling, packaging, and sealing
4. Sample cooling
5. Keeping samples in liquid nitrogen
6. Refreezing to utilise Advantages of Preservation

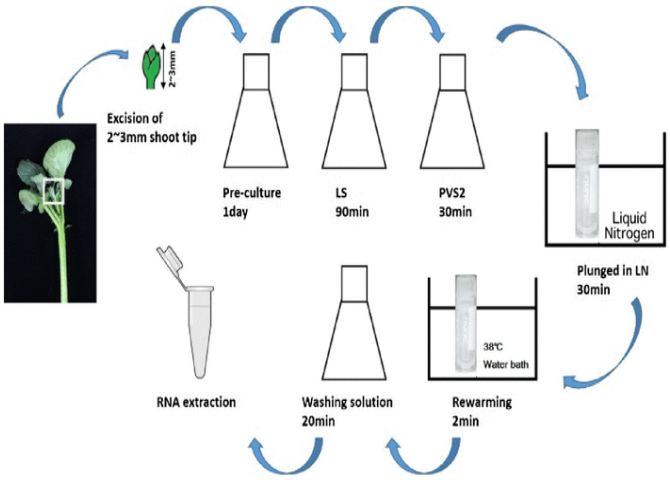

Fig. 1 Schematic Diagram of Cryopreservation

Benefits of Cryopreservation

There are many benefits of cryopreservation technique. These include:

- ❖ Treatments for fertility.
- ❖ Little labour and space are needed.
- ❖ Protection against genetic taint.
- ❖ Preserves the genetic integrity of priceless stains.
- ❖ Protects endangered species' germplasm.
- ❖ It is possible to maintain biological samples for an extended length of time.
- ❖ Shields the samples from microbiological and medical contamination.
- ❖ Uses cryopreservation to stop genetic drift in gametes, embryos, etc.

Types of Plant Materials Subjected to Cryopreservation

Algae, bryophytes, ferns, dedifferentiated cell cultures, embryogenic cultures, excised embryos and embryonic axes, latent buds, meristem and shoot tips, root tips, pollen, and seeds can all be preserved using cryopreservation procedures now accessible (Nukari et al., 2009).

Application of Germplasm Cryopreservation Technology

One long-term storage method called cryopreservation is primarily used to keep biological samples viable for an extended period of time. Numerous fields, such as cryosurgery, molecular biology, ecology, food science, plant physiology, and numerous medical applications, use this preservation technique extensively. Additional uses for the cryopreservation method include:

1. Gene Bank.
2. Blood transfusion.
3. In vitro fertilization.
4. Organ transplantation.
5. Artificial insemination.
6. Storage of rare germplasm

Conclusion

Since the use of cryopreservation facilitates the storage and quick multiplication of plant germplasm in a pathogen-free aseptic environment as well as the optimisation of physical space and labour, the initial technological investment for the practice will be lower than the maintenance costs for applying the various techniques later on. When it comes to conservation strategy, it is more expensive to introduce an accession into cryopreserved storage than it is to establish one in the field or in vitro culture. However, cryopreservation costs are significantly lower for long-term (more than 20 years) maintenance in the field or in vitro, especially when numerous accessions are preserved.

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