An Introduction to Germplasm and Cryopreservation

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Introduction

- **Germplasm**

- A germplasm is a collection of genetic resources for an organism.

- Germplasm is the genetic material of an individual that may be transmitted, sexually or somatically, from one generation to another.

- Plant or animal material (such as seeds, pollen, rootstock, or sperm) that is collected and stored chiefly for future use in breeding, conservation, or research.

Conservation of Germplasm

- **In-situ preservation**
- Preservation of the germplasm in their natural environment by establishing biosphere, national park.

- **Ex-situ preservation**
- In the form of seeds or invitro culture

In-situ conservation

- **Advantages**
- Plants and animals conserved in their natural environment.
- Biodiversity permanently protected.
- Representative examples of ecosystems also permanently protected.
- Natural and cultural heritage protected permanently.
- Ecological integrity is maintained and managed.
- **Disadvantage**
- Endangered habitats may be fragmented so the area may not be large enough to ensure the survival of these species.
- Genetic diversity may have already been dramatically decreased.
- Conditions that threatened the organisms in the area may still be present, e.g. disease or interspecific competition.

Ex-situ Conservation

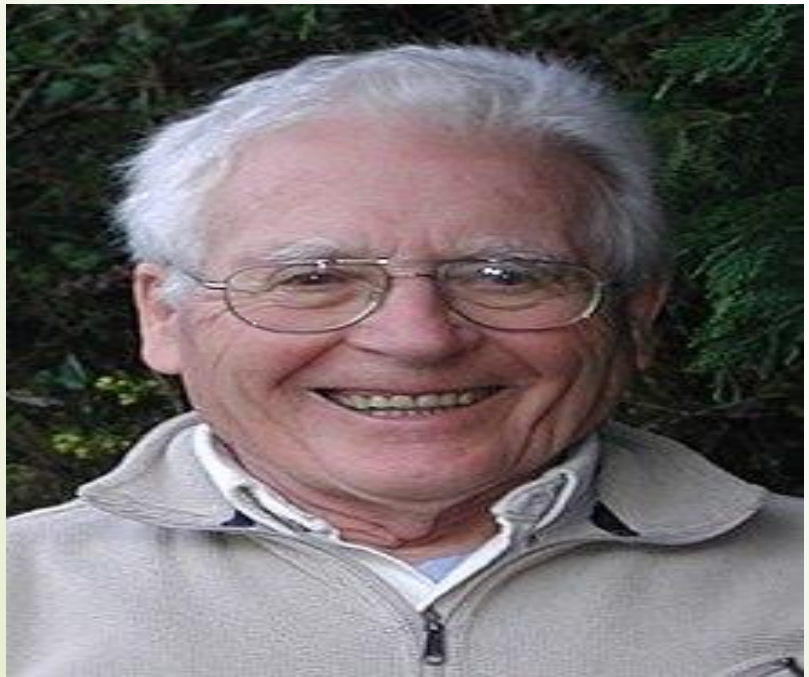
- **Advantages**
- Organisms are completely protected from predation and poaching
- Health of individuals can be monitored and medical assistance given as required
- Populations can be more effectively managed and divided if disaster strikes
- Genetic diversity of the population can be measured
- Selective breeding programs can be put into place
- **Disadvantages**
- Captive population have limited genetic diversity
- Animals can be exposed to a wide range of different diseases
- the organisms are living outside their natural habitat
- Nutritional issues may arise
- Animals may not behave as normal making reproduction difficult

Cryopreservation

- Cryopreservation is a non lethal storage of biological material at ultra –low temperature.
- Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues.
- At a temperature of liquid nitrogen (-196c) almost all metabolic activities are ceased and the sample can preserved in such state for extended periods.

HISTORY

- One early theoretician of cryopreservation was **James Lovelock**. In 1953, he suggested that damage to red blood cells during freezing was due to osmotic stress.
- In the mid-1950s he experimented with the cryopreservation of rodents.
- Cryopreservation was applied to human materials beginning in 1954 with three pregnancies resulting from the insemination of previously frozen sperm.
- Fowl sperm was cryopreserved in 1957 by a team of scientists in the UK directed by **Christopher Polge**.



Steps of cryopreservation

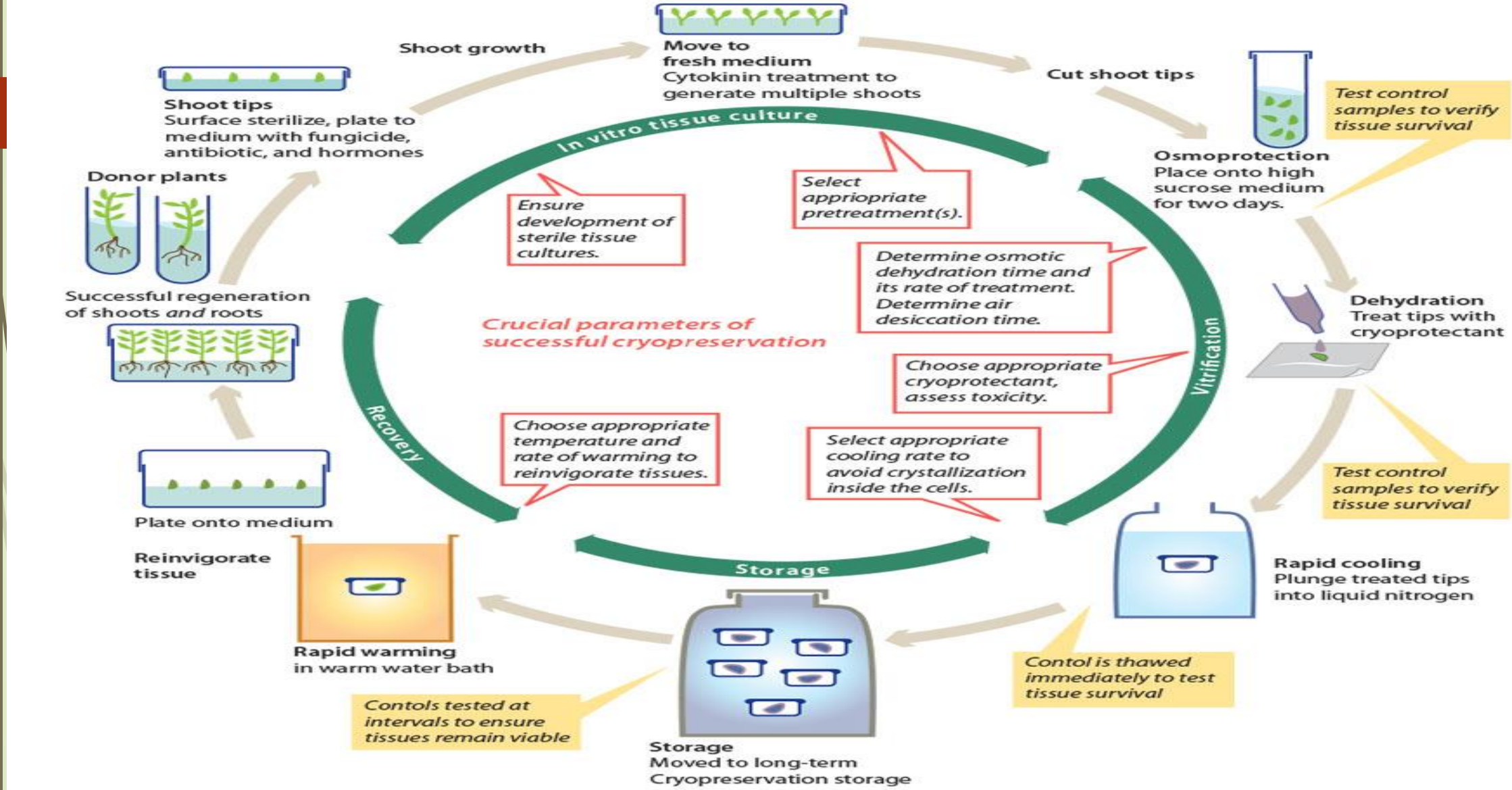
The technique followed by the regeneration of plants involves the following steps.

- **Selection of Material:** the selection of proper plant material is important.
- **Addition of Cryoprotectants:** The chemical material is important as it prevents cryo destruction. Eg alcohol, proline dimethyl sulfoxide.
- **Freezing:** Different species of plants show different types of sensitivity to low temperatures. They are different types of methods.

- **Slow Freezing Method-** In this process, the tissue or plant material is slowly frozen at a slow cooling rate.
- **Rapid Freezing Method -** The vials are plunged in liquid nitrogen.
- **Dry Freezing Method -** In this method hydrated cells and seeds are stored.

Steps of cryopreservation

- **Storage in Liquid Nitrogen:** It is also important for the maintenance of the sale or material at a specific temperature. (to - 196°C.)
- **Thawing:** The thawing process is usually carried out by plunging the vials into a warm water bath with vigorous swirling. It also causes the vials to get transferred or move to another bath at 0 °C
- **Washing & Reculturing:** The preserved material is washed to remove the Cryoprotectants. Furthermore, the material is recultured in a fresh medium.
- **Measurement of Viability:** Due to storage stress, there is a possibility of cell death. The presence of viability can be seen in most cases.
- It is calculated by the formula:
(no of cells growing/no of cells thawed)×100
- **Regeneration of Plants:** After that, the viable seeds are cultured on a non-specific growth medium. Suitable environmental conditions are maintained.


Shoot growth
Move to fresh medium
Cytokinin treatment to generate multiple shoots
Cut shoot tips
Test control samples to verify tissue survival
Shoot tips
Surface sterilize, plate to medium with fungicide, antibiotic, and hormones
In vitro tissue culture
Osmoprotection
Place onto high sucrose medium for two days.
Donor plants
Ensure development of sterile tissue cultures.
Select appriopriate pretreatment(s).
Determine osmotic dehydration time and its rate of treatment. Determine air desiccation time.
Dehydration
Treat tips with cryoprotectant
Successful regeneration of shoots and roots
Crucial parameters of successful cryopreservation
Choose appropriate cryoprotectant, assess toxicity.
Vitrification
Recovery
Choose appropriate temperature and rate of warming to reinvigorate tissues.
Select appropriate cooling rate to avoid crystallization inside the cells.
Test control samples to verify tissue survival
Plate onto medium
Reinvigorate tissue
Storage
Rapid cooling
Plunge treated tips into liquid nitrogen
Rapid warming
in warm water bath
Contol is thawed immediately to test tissue survival
Contols tested at intervals to ensure tissues remain viable
Storage
Moved to long-term Cryopreservation storage

Merits of Cryopreservation

- Effective means to conserve the germ plasm of endangered species.
- Fertility preservation.
- Methods to reduce multiple pregnancies
- Larger range of stocks available.
- Easy disease- free exchange of stocks, nationally and internationally.
- Stocks remain viable indefinitely.
- Safety from disease, genetic contamination and breeding failure.

Demerits of Cryopreservation

- Does not work efficiently for all strains
- Formation ice crystals inside the cells cause injury to the organelles and the cell.
- High intracellular concentration of solutes can be very damaging to cells.
- Sometimes, certain solutes from the cell may leak out during freezing.
- Cryoprotectants also affect the viability of cells.
- Migration of water, causing extracellular ice formation, can also cause cellular dehydration. The associated stresses on the cell can cause damage directly.
- The physiological status of the plant material is also important.

THANK YOU