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FACULTY OF ENGINEERING & TECHNOLOGY

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LECTURE 6

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Cryopreservation

Cryopreservation (Greek,krayos-frost)literally means in the frozen state. The principle involved in cryopreservation **to bring the plnt cells and tissue cultures to a zero metabolism or non-dividing state by reducing the temperature in the presences of cryoprotectants** (DMSO (dimethyl sulfoxide), glycerol, ethylene, propylene, sucrose, mannose, glucose, praline, acetamide etc).

CRYOPRESERVATION broadly means the storage of germplasm at very low temperature using :-

- ✓ Over solid carbon dioxide(at 79°C)
- ✓ Low temperature deep freezer(at -80°C)
- ✓ Using vapour nitrogen (at- 150°C)
- ✓ In liquid nitrogen(at -196°C)

Among these, the most commonly used cryopreservation is by employing liquid nitrogen. At the temperature of liquid nitrogen(at -196°C), the cell stay in a completely inactive state and thus can be conserved for longer period. Infact cryopreservation has been successfully applied for germplasm conservation of some plant species e.g rice, wheat, peanut, sugarcane, coconut.

MECHANISM OF CRYOPRESERVATION

The technique of freeze preservation is based on the **transfer of water present in the cells from a liquid to solid state.** Due to the presence of salts and organic molecules in the cells, the cell water requires much more lower temperature to freeze(even up to -68°C) compared to the freezing point of pure water(around 0°C). **When stored at low temperature, the metabolic processes and biological deteriorations in the cells/tissues almost come to standstill.**

TECHNIQUE OF CRYOPRESERVATION

The cryopreservation of plant cell culture followed the regeneration of plants broadly involves the following stages.

1. Development of sterile tissue culture.
2. Addition of cryoprotectant and pretreatment.
3. Freezing
4. Storage
5. Thawing
6. Reculture
7. Measurement of survival/viability
8. Plant regeneration

1.DEVELOPMENT OF STERILE TISSUE CULTURE

The selection of plant species and the tissue with particular references to the morphological and physiological characters largely influences the ability of the explants to survive in cryopreservation. Any tissue from a plant can be used for cryopreservation e.g. meristems, embryos, endosperm, ovules, seeds, culture plants.

2.ADDITION OF CRYOPROTECTANT

Cryoprotectant are the compound that can **prevent the damage caused to cells by freezing or thawing**. There are several cryoprotectant which include:

(DMSO, GLYCEROL, ETHYLENE, PROPYLENE, SUCROSE, MANNOSE, GLUCOSE…..)

3.FREEZING

The sensitivity of the cells to low temperature is visible and largely depends on the plant species.Four different types of freezing are used. Slow freezing method Rapid freezing method Stepwise freezing method Dry freezing method

4.STORAGE

Maintenance of the frozen cultures at the specific temperature is as important as freezing. In general, the frozen cells/tissues are kept for storage at temperature in the range of -72 to-196°C. **Storage is ideally done in liquid nitrogen refrigerator at - 150°C in the vapour phase, or at -196°C in the liquid phase.** The ultimate objective of storage is to stop all the cellular metabolic activities and maintain their viability. For long term storage temperature at -196°C in liquid nitrogen is ideal.

5.THAWING

Thawing is usually carried out by plunging the frozen sample in ampoules into the warm water (temp 35- 45°C) bath with vigorous swirling. By this approach,rapid thawing(at the rate of 500-750°Cmin-1)occurs, and this protects the cell from the damaging effects ice crystal formation. As the thawing occurs (ice completely melts) the ampoules are quickly transferred to a water bath at temperature 20-25°C. This transfer is necessary since the cells get damaged if left for long in warm(35-45°C) water bath.

6.RECULTURE

In general thawed germplasm is washed several times to remove cryoprotectant. The material is then cultured in a fresh media.

7.PLANT REGENERATION

The ultimate purpose of cryopreservation of germplasm is to regenerate the desired plant. For appropriate plant growth and regeneration, the cryopreserved cell/tissue have to be carefully nursed and grown. Addition of certain growth promoting substances ,besides maintenance of appropriate environmental condition is often necessary for successful plant regeneration.

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