Cryopreservation – A technique to store plant cells and tissues for germplasm conservation

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Germplasm storage : Germplasm is the sum total genetically variable stocks of a plant species. The maintenance of genetic stocks in living condition for a long time is known as germplasm storage or germplasm maintenance or germplasm conservation. Germplasm provides the raw materials, which the breeder uses to develop commercial crop varieties. Conventionally germplasm are stored in the form of seeds because they occupy a relatively small space and can be stored for many years. But there are a number of important vegetatively propagated crops like potato, sweet potato, cassava etc. where conventional method is not applicable. It is now possible with modern tissue culture techniques to provide a germplasm storage procedure which uniquely combines the possibilities of disease elimination and rapid clonal propagation.

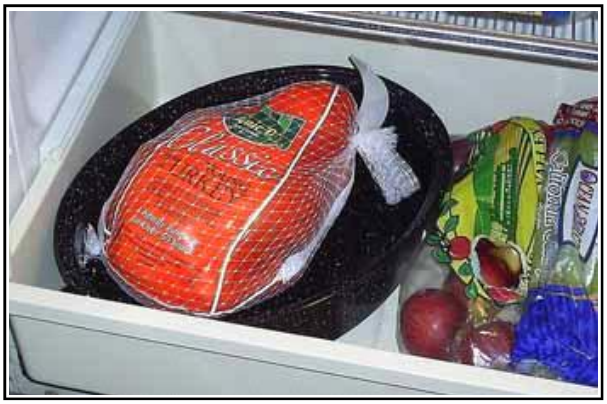
Some techniques involve storage in growing stage where as others relate to the suspension of growth. Growth inhibition may be by temperature reduction, use of growth retardant chemicals or hormones, reduction in oxygen concentration etc. these methods require periodic renewal after some intervals. Another method is cryopreservation which often can store plant materials for virtually indefinite periods. This technique preserved original genetic stocks in a limited area and ensures genetic stability of cryopreserved material.

Cryopreservation : The preservation of cells, tissues and organ in liquid nitrogen (at -196^{0}C) is called cryopreservation and the science pertaining to this activity is known as cryobiology. Cell suspensions, hybrid protoplasts, pollen grains, seeds and meristems of desired plant species are stored in this method for the establishment of germplasm bank. Liquid nitrogen maintains low temperature for the long term storage. The low temperature reduces the growth rate of the stored cells and delays ageing. Therefore, the plant materials do not lose their viability for a long time. After a long period of storage plants can be regenerated from the cryopreserved plant materials.

National Bureau of Plant Genetic Resources (NBPGR), New Delhi has this facility. Bureau increased its storage capacity from 154,964 varieties to 600,000 varieties. The expansion project has been promoted by the realization that the country's genetic diversity is being steadily eroded by modern agricultural practices, urbanization and deforestation.

The essential steps for cryopreservation of plant materials are as follows:

Freezing and storage of plant materials: It may be either slow (cooling rate 1^{0}C to 4^{0}C per min) from 0^{0}C to -100^{0}C and then transfer to liquid nitrogen or it may be initially slow (5^{0}C per min) up to -30^0 C to -50^{0}C, held at this temperature for about 30 min and then cooled rapidly by plunging the material vials into liquid nitrogen. A partial dehydration of the material before freezing has been found to increase the survival of cells/ tissues. The preserved may also be encapsulated in matrices like alginate before freezing. A cryoprotectant must be added to the culture medium to protect the cells from injury. Cryoprotective agents are chemicals which protect plant materials from damaging effects of cold storage. They work as anti-freezing agents. They prevent the formation of large crystals in the cells. A mixture of Dimethylsulfoxide (DMSO), glycerol and proline is quite suitable as cryoprotectant. This pretreatment makes the material hardy and improves their survival. The cell culture is transferred to a small flask or beaker. The flask is insulated with a thick plastic cover to prevent damages by liquid nitrogen. The culture flask is then kept in liquid nitrogen in a container. The container is kept in a refrigerator. The liquid nitrogen keeps the temperature -196^{0}C inside the culture vessel. At this temperature there is no metabolic activity in plant cells due to inactivation of cellular enzymes. This material can be stored for five years



or more.

Thawing: Thawing of the frozen material is achieved by plunging the vials into water at 35^{0}C to 40^{0}C for 90 sec. Thawing melts extra cellular ice crystals formed during the long term storage. Immediately after melting of ice crystals the culture flask is kept in a water bath.

Removal of cryoprotectants: The stored plant cells have cryoprotectants on the surface of cell walls. The cryoprotectants interfere with growth of the cells in callus and plant regeneration. Generally it is washed with distilled water. The cells are transferred to a nutrient medium and incubated at 25^{0}C for callus induction.

Reculture: Reculture means production of plantlets from the cryopreserved plant materials. Cryopreserved plant material may show some special requirements during reculture, like GA3 treatment for the reculture of shoot tips of tomato, treatment of activated charcoal or the reculture of carrot plantlets etc. generally meristematic cells survive better than mature differentiated cells during cryopreservation.

Achievements:

– Cryopreserved protoplasts of carrot have been successfully regenerated.

– Meristems of potato, cassava, sugarcane, peanuts, etc. are stored for several years.

– Cell suspensions of soybean, carrot, datura etc. are stored for two to five years.

– Pollen grains and protoplast hybrids of peanuts, mustard, cotton, wheat, rice etc. can be stored for several years.

Scientists of the world over are availing of the NBPGR seed facility through germplasm exchange program. The export of few plant species is banned like black papper in the national interest and to protect India's supremacy in the international trade. Within India, anyone involved in plant or crop research can get seeds/germplasm from the NBPGR.

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