Germplasm Conservation

DR. NISHI MATHUR

The sum total of all the genes present in a crop and its related species constitutes its germplasm. It is ordinarily represented by a collection of various strains and species. Germplasm provides the raw .materials (= genes). The breeder uses these to develop commercial crop varieties.
Therefore, germplasm is the basic indispensable ingredient of all breeding programmes. Thus a great emphasis is placed on collection, evaluation and conservation of germplasm. Conventionally, germplasm is conserved as seeds stored at ambient temperature, low temperature or ultralow temperature.

Applications or significance of germplasm conservation

1. **The conservation of germplasm involves the preservation of the genetic diversity of a particular plant or genetic stock. It can be used at any time in future.**
2. **It is important to conserve the endangered plants or else some of the valuable genetic traits present in the existing and primitive plants will be lost.**
3. **Main crops produce recalcitrant or short lived seeds.**
4. **Similarly, in case of clonal crops seeds are not the best material to conserve due to their genetic heterogeneity and unknown worth. Their genes need to be conserved.**
5. **The roots and tubers loose viability rapidly. Their storage requires large space, low temperature and is expensive.ft. In addition, materials modified by genetic engineering may some, times be unstable. Such materials are needed to be conserved intact for future use.Methods of gerniplasm conservationA global organization- International Board of Plant Genetic Resources (IBPGR) has been established for germplasm conservation and provides necessary support for collection, conservation and utilization of plant genetic resources through out the world. The germplasm is preserved by the following two ways:(a)In-situ conservation**
   The germplasm is conserved in natural environment by establishing biosphere reserves such as national parks, sanctuaries. This is used in the preservation of land plants in a near natural habitat along with several wild types.

   (b)Ex-situ conservation
   This method is used for the preservation of germplasm obtained from cultivated and wild plant materials. The genetic materials in the form of seeds or in vitro cultures are preserved. It is stored as gene banks for long term use. There are two types of gene banking.

   - ***In vivo* gene banks have been made to preserve the genetic resources by conventional methods e.g. seeds, vegetative propagules, etc.**
   - ***In vitro* gene banks have been made to preserve the genetic resources by non – conventional methods such as cell and tissue culture methods. This will ensure the availability of valuable germplasm to breeder to develop new and improved varieties.**

   In vitro conservation of germplasm

The methods involved in the'in vitro conservation of germplasm arc: (a) Cryopreservation In eryopreservation (Greekkrayos-frost)

In this case the cells are preserved in the frozen state. The germplasm is stored at a very low temperature using

- **Solid carbon dioxide (at -790C)**
- **Using low temperature deep freezers (at -800C)**
- **Using vapour nitrogen (at- 1500C)**
- **Liquid nitrogen (at-1960C).**

The cells stay in completely inactive state. Thus they can be conserved for long periods. Any tissue from a plant can be used for cryopreservation e.g. meristems, embryos, endosperms, ovules, seeds, cultured plant cells, protoplasts, calluses. Certain compounds like- DMSO (dimethyl sulfoxide), glycerol, ethylene, propylene, sucrose, mannose, glucose, praline, acetamide etc are added during the cryopreservation. These are called cryoprotectants and prevent the damage caused to cells (by freezing or thawing) by reducing the freezing point and super cooling point of water.

(b) Cold Storage

Cold storage is a slow growth germplasm conservation method. It conserves the germplasm at a low and non-freezing temperature (1- 9°C). The growth of the plant material is slowed down in cold storage in contrast to complete stoppage in cryopreservation. Thus it prevents cryogenic injuries. Long term cold storage is simple, cost effective. It yields germplasm with good survival rate. Virus free strawberry plants could be preserved at 10°C for about 6 years. Several grape plants have been stored for over 15 years by using a cold storage at temperature around 9°C and transferring them in the fresh medium every year.

(c) Low pressure and low oxygen storage

In low- pressure storage, the atmospheric pressure surrounding the plant material is reduced. In the low oxygen storage, the oxygen concentration is reduced. The lowered partial pressure reduces the in vitro growth of plants. In the low-oxygen storage, the oxygen concentration is reduced and the partial pressure of oxygen below 50 mmHg reduces plant tissue growth. Due to the reduced availability of 0_2, and reduced production of CO_2, the photosynthetic activity is reduced. It inhibits the plant tissue growth and dimension. This method has also helped in increasing the shelf life of many fruits, vegetables and flowers.

The germplasm conservation through the conventional methods has several limitations such as short-lived seeds, seed dormancy, seed-borne diseases, and high inputs of cost and labour. The techniques of cryo-preservation (freezing cells and tissues at -1960c) and using cold storages help us to overcome these problems.

Advantages and Limitations of Germplasm Storage

The in vitro techniques for germplasm storage have great advantage for clonally propagated crops and for crops having recalcitrant/short- lived seems. The potential advantages of these techniques are as follows:

(i)Requirement of relatively very small space,

(ii)Storage of germplasm free from diseases, insects and other pathogens, and weeds,

(iii) Storage over long periods,

(iv) Reduced risk of errors in labeling, etc. In addition, such materials are 'clean' sources of 'nucleus seed',

(v)They are ideal for germplasm exchange.

However, these approaches suffer from the following disadvantages:

(i) Sophisticated facilities are required (particularly for freeze preservation and DNA cloning),

(ii) They demand a greater skill in handling and maintenance than the conventional techniques,

(iii)Even shoot tip derived plants may show genetic instability. *. at least in some plant species.
(iv) Cells/tissues become damaged during cryopreservation.

(v)Even DNA may become damaged due to cryopreservation under suboptimal conditions.

(d) Slow Growth Cultures
Slow-growth of plantlets ill vitro provides an attractive alternative to freeze preservation of germplasm as it is simpler, cheaper and very effective. Slow growth may be achieved by maintaining the plantlets either at a low temperature (4-9°C or Ca. I5°C) or on a medium having high osmotic concentration (e.g., 20% sorbitol or sucrose) or both.

In addition, the nutritional status of the medium may be lowered to restrict the growth of plantlets. Under the conditions of slow-growth, cultures may be attended to only once in several months. Its subculture may, be necessary only after long periods, once every ¶236 months.

For example, grape plantlets stored at 9° C need to be transferred after 12 months; they have been thus maintained for more than 15 years. It has been estimated that 6 replicates of 800 grape varieties can be maintained in only 2 m2 laboratory space, while it would require I ha in the field.

The slow-growth approach is being utilized for germplasm conservation of specified root, tuber and tree species by the NBPGR, New Delhi. A National Facility for Plant Tissue Culture Repository has

been created for this purpose. It has so far developed the slow-growth protocols for ginger, garlic, banana, sweet potato, etc.

(e) Desiccated Somatic Embryos (SE) and Artificial Seeds

The techniques for desiccation of SE s and for production of desiccated, artificial seeds are now becoming available. The desiccated SE s and artificial seeds can be stored at low (4°C) or ultralow (-20°C) temperatures for prolonged periods in a manner similar to zygotic seeds.

This approach is yet to be evaluated for such an application. It involves complicated desiccation procedures. It can be applied only to those species/genotypes where SE regeneration occurs in adequate frequency.

(f)DNA Clones

Germplasm can also be conserved in form of DNA segments cloned in a suitable vector, e.g., cosmids, phasmids or YACs. The technique is highly sophisticated, technically demanding and expensive.

It is likely to be used for conservation of valuable genes or DNA segments from threatened species. It can also be used for the conservation of the entire genomes of various germplasm lines of different species.

Comparison of Three Approaches for In Vitro Germplasm Conservation

Feature	Cryopreservation	Slow-growth	DNA clones
Tissue/organ conserved	Shoot-tips, zygotic or somatic	slow growing shoots	DNA pieces as phage clones
Metabolic activity	Nil	Slow	Nil
Storage temperature	-196°c	4-9 0r 15°	4°C in lyophilized state
Storage in	Liquid nitrogen refrigerators	ordinary refrigerators	Deep freeze
Attention needed during storage	Replenishing liquid nitrogen	subculture every 6-36 months	virtually nil