Agro Genetic Resources Conservation

Phytosanitary aspects of plant germplasm conservation. Cryopreservation. Cataloging, characterization, evaluation and utilization of genetic resources. National seed policy, Seed: seed health, diseases, quality, viability and storage

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Meaning of agro biodiversity

• The subset of biodiversity related to agriculture is known as agro biodiversity. It is defined as the diversity of the agriculture systems, all agriculture-related species and individuals within species.

• It includes all flora and fauna, plants, animals, birds, reptiles, amphibians, insects, soil organisms, and other agriculture production. These also include the wild related species of the cultivated plants and the domesticated animals as they have the potential to provide donor genetic stocks for various important traits.

• It is a basis of stability in agricultural production, livelihood of farming households and communicates, sustainable development, and resources for further development of improved strains.

• It gives farming systems the opportunity and means for nutrient recycling, maintaining good soil conditions.

• A rich agro biodiversity is could provide both social-economic and ecological benefits and thus it is important to both agriculture and society.

• Another underlying importance of agro biodiversity is that it provides genetic resources of actual or potential value for further improvement in agriculture productivity and production.
Agro Genetic Resources

• Diverse genetic resources are the foundation for sustainable development of agriculture.

• Global food security always relies on intelligent use of crop genetic resources which contain the essential building blocks that are critical to food security.

• Their availability is a fundamental requirement for achieving further productivity increase and higher nutritional values through plant breeding.

• For this, it is crucial to conserve the existing crop diversity and to allow agricultural researchers, breeders and farmers access to it.
Indicators of agro biodiversity

• The distribution of diversity in the agro ecosystems, between species and within species has a direct, system wide relevance. Thus, in a way, the occurrence of diversity of the agro ecosystem has led to enhancement of overall agro biodiversity because of the wide and/or differential adaptation of the same and related specie across these agro ecosystems, thereby enriching the other components of agro biodiversity.

• The agro ecological evidences suggest that the agriculture has been practice in Nepal since ancient times. This fact also substantiates that human intervention over a long period has led to development and stabilization of crop diversity in the country.

• The natural or disturbed occurrence of wild relatives of wild relatives of crop plants in different agro-climate zones of the country could provide a key indicator in this regard.

• Further, it may be difficult to substantiate this diversity in the cultivation zones but the traditional agro ecosystems, also termed as the ethnic agro ecosystems, provide positive indicators.
Agro Genetic Resources

- **Three agro-ecological zones of Nepal** (Tarai, Mid Hill and High Hill) experience a wide range of climate from tropical to temperate and arctic.

- The variation is mainly attributed to immense changes in elevation with the greatest range of altitude on Earth, from 60 to 8848 masl.

- Out of about **410 angiosperm families in the world**, 203 (almost 50%) are represented in Nepal.

- The Biodiversity Profiles Project (1995) ranked **Nepal** as having the **tenth richest flowering plant diversity in Asia**.

- On a world scale, Nepal is placed in 31st. More than 500 species of edible genetic resources are available, of which nearly 200 species are under cultivation (Upadhyay and Joshi 2003).
Sustainable use of agro biodiversity

• Genetic diversity is important for three major reasons. First, it helps in providing stability of farming systems through a range of inter-and intra-specific characteristics. Secondly, it provides insurance against changing environmental conditions that may be anticipated for the future. Thirdly, it embodies characteristics that are potentially valuable, but not yet exploited.

• The genetic diversity also helps to withstand changes in environment, climate and agro ecosystem besides the treats from pests and diseases.

• The agro biodiversity exists primarily at the intra-species level and is important in respect of the genetic variability therein. New varieties of traditional crops continue to be developed through traditional and modern tools.

• The new plant varieties will have to be well adapted to harsher environment conditions, such as stress or excess of temperature, moisture and salts, in order to ensure overall growth in a agricultural productivity and production.

• The sustainability of agricultural systems rather than their short-term productivity will have to be ensured besides minimizing/mitigating the other negative side effects of modern agriculture.
Concern for loss of agro biodiversity

- Natural habitats of crop plant relative and traditional/ethnic agro-ecosystems are squeezing, and also losing the diversity therein, due to the expansion of agricultural production to frontier areas. Diversity is being threatened/lost from the agricultural field as well due to the adoption of improved varieties and other technologies by the farmers.

- Agro ecosystem have been losing diversity due to factors such as (i) a heavy use of agro chemicals, (ii) uniform crops/varieties over the time and the space minimizing the synergistic equations with the diverse soil biota, (iii) heavy tillage with farm machinery disrupting the soil structure, and (iv) declining use of animal manure, crop residues intercropping, cover crops, crop rotation and other farm practices that would maintain/increase the organic matter content of soils and reduce their exposure to the forces of erosion. These factors play together to affect losses in soil and crop productivity.

- Use of agro chemicals, particularly the pesticides, is an important concern. This is so because besides managing the target pests, it also leads to the killing of beneficial insects.
Agro Genetic Resources Conservation

• About half of the average global production increase in cereals that were achieved under the Green Revolution was attributable to plant breeding utilizing plant genetic resources.

• The role of crop diversity and plant breeding will become even more important in the near future for achieving food security in a sustainable way.

• Plant genetic resources that are conserved in the Genebank can be used for:
  – Safe conservation for future use
  – Direct use for agricultural production
  – Conservation of diversity in environment
  – Scientific use for experimental materials
  – Genetic enhancement (pre-breeding)
  – Breeding materials for the sustainable development
Agro Genetic Resources Conservation

- According to the Food and Agriculture Organization of the United Nations (FAO), more than 75% of global crop diversity has disappeared irrevocably over the 20th century (1900 to 2000).

- A large number of wild relatives of important food crops are also likely to disappear over the next decades due to climate change.

- Realizing the significance of agricultural genetic resources in national development, the Government of Nepal and Nepal Agricultural Research Council (NARC) has established the National Agriculture Genetic Resources Center (Genebank) in 2010 for conservation and utilization of agro-biodiversity.
Agro Genetic Resources Conservation

- Agro-biodiversity has a significant role for the food security and livelihood of human beings.

- Since time immemorial, our ancestors have been conserving, maintaining and developing this diversity.

- In an agro-based country like Nepal, where agrobiodiversity is the backbone for the sustainable development of agriculture, food security and poverty alleviation, it is the national responsibility to conserve, maintain and sustainable use of the available diversity.

- This necessitates effective institutional environments and programs to meet the needs and aspirations of the future generations.
Agro Genetic Resources Conservation

• A total of 2.4 ha is allocated for this Center and additional one ha is available for field experiment near the center.

• Out of 2.4 ha, Genebank building has occupied 0.092 ha, 0.26 ha is allocated for Field Genebank and 0.83 ha for regeneration, multiplication, characterization, evaluation and post quarantine activities. Residential building (for curators) is in about 0.15 ha.

• Other existing supplementary mechanisms for conserving agro-biodiversity in Nepal are
  – Ritual practices of Hindu (Rudrakshya, Tulsi, Jau, etc).
  – Culturally protected areas (temple and other religious places).
  – Government’s protected areas
  – National parks, leasehold, community and private forests
  – Rangeland and wetland management system,
  – Farmers seed network system, and
  – Protection of some plant species.
Genetic Resources that are Conserved

- Landraces
- Modern varieties
- Obsolete varieties
- Breeding lines, RILs, Genetic stocks, NILs, Differential lines
- Exotic genetic resources
- Crop wild relatives
- Wild edible plants
Total Crops Accessions Conserved Genebank

- Cereals: 6000
- Millets: 750
- Pulses: 1800
- Oilseeds: 185
- Vegetables: 565
- Field Gene Bank: 310
- Tissue Bank: 10
- Others: 1500
- Total: 11120
Phytosanitary aspects of plant germplasm conservation

• Germlasm broadly refers to the **hereditary material (total content of genes)** transmitted to the offspring through germ cells.

• Germlasm provides the **raw material** for the breeder to develop various crops. Thus, conservation of germlasm assumes significance in all breeding programmes.

• The very objective of germlasm conservation (or storage) is **to preserve the genetic diversity of a particular plant or genetic stock** for its use at any time in future.

• In recent years, many new plant species with desired and improved characteristics have started replacing the primitive and conventionally used agricultural plants. It is important to **conserve the endangered plants** or else some of the valuable genetic traits present in the primitive plants may be lost.
Conservation Strategies

- Gene bank has adopted the following conservation strategies, which are only possible in collaboration with all stakeholders for exploration and collection, regeneration, multiplication, characterization and evaluation, and safety duplication.
  - Ex-situ conservation
    - Seed Conservation as base and active collections (Seed bank)
    - In-vitro Conservation (cold storage and cryopreservation) (Tissue bank)
    - DNA bank
    - Field Gene bank
  - On-farm Conservation
  - In-situ Conservation
On-Farm Conservation

• It is a dynamic conservation of local and important crop varieties.

• Genebank has been supporting on-farm conservation since 2001 by involving farmers and their genetic resources in researches, by establishing Community Seed Bank in Kachorwa, Bara (2003) and Simariya, Sunsari (2011) and by enhancing landraces.

• It is initiated to strengthen the Dalchowki CSB, first of its kind in Nepal established in 1994. Diversity fairs and diversity blocks are the major activities to collect and maintain the varietal diversity in CSB.
In-Situ Conservation

• The conservation of germplasm in their natural environment by establishing biosphere reserves (or national parks/gene sanctuaries) is regarded as in-situ conservation.

• This approach is particularly useful for preservation of land plants in a near natural habitat along with several wild relatives with genetic diversity.

• The in-situ conservation is considered as a high priority germplasm preservation programme.

• Limitations of in-situ conservation:
  – The risk of losing germplasm due to environmental hazards
  – The cost of maintenance of a large number of genotypes is very high.
In-Situ Conservation

• This is for wild edible plants and wild relatives of cultivated crop species.

• The sites where important wild edible plants and wild relatives exist, is planned to conserve in collaboration with National Parks, religiously and culturally protected sites, heritage sites and communities.

• It needs to locate species that needs to be conserved and develop strategies to protect their habitat collaborating with relevant stakeholders.
Ex-Situ Conservation

• Ex-situ conservation is the chief method for the preservation of germplasm obtained from cultivated and wild plant materials.

• The genetic materials in the form of seeds or from in vitro cultures (plant cells, tissues or organs) can be preserved as gene banks for long term storage under suitable conditions.

• For successful establishment of gene banks, adequate knowledge of genetic structure of plant populations, and the techniques involved in sampling, regeneration, maintenance of gene pools etc. are essential.
TYPES OF *EX SITU* COLLECTIONS

Four types of *ex situ* germplasm collections are recognized based on the duration and importance of conservation:

1. Base Collections
2. Active Collections
3. Working Collections
4. Core Collections
• **Ex-Situ Conservation (Seed Bank) for Orthodox Seeds**
  
  **Base collections (original collections):**
  
  – Collections are stored at -18 °C with a relative humidity of 40% for 50 to 100 years. Seed moisture is lowered to 3 to 7% depending on crop species.
  
  – Also called **long term storage system**.
  
  – Size of accession is about 2000 seeds for self-pollinated crops and 4000 seeds for cross-pollinated crop species.

• **Active collections**:
  
  – Collections are stored at 5 to 10 °C with a RH 30 to 40% for 5 to 15 years.
  
  – Also called **short term storage system** and used to characterize, evaluate, multiply and distribute.
  
  – Accessions size is about 4000 seeds for self-pollinated crops and 8000 for cross pollinated.
• **Working Collections:**
  – Collections under short-term storage (3-5 years)
  – Maintained at 5-10 °C temperature with 8-10% moisture content.
  – Breeders’ collections that are utilized for different breeding purposes.

• **Core Collections**
  – Includes the entire genetic diversity of a species conserved with minimum replications.
  – Represents a subset of the entire germplasm with all useful characters so that identification of useful entry becomes easy and accessible to breeders.
• **Field Gene bank (In vivo conservation)**
  - Essential for those crop species having **recalcitrant seeds** and vegetatively propagated and apomictic crop species for conservation, characterization, evaluation and utilization.
  - Government’s farm, around the road and office buildings, community farms, botanical garden, culturally protected and heritage sites are suitable for Field Genebank.

• **In-Vitro Conservation (Tissue Bank)**
  - Very effective for conserving those crop species, which either produce recalcitrant seeds or does not produce any seeds.
  - Can conserve many samples in small areas and conserved materials develop very slowly mainly due to nutrient depletion and low temperature.
  - Such type of materials can be multiplied rapidly and can easily be kept free from viruses, insect parasites, fungi or bacteria.
  - Cultures can be kept in test tubes on nutrient medium for indefinite periods of time by transferring at regular interval.
Material used for Conservation

- The materials stored in vitro may be **protoplasts, cells from suspension or callus cultures, meristem tips, propagules** at various stages of development or organized plantlets.

- The **storage of germplasm by repeated cultures** has some disadvantages which are **risk of material loss** due to human errors or the failure in maintenance of in vitro security resulting in invasion by the pathogen.

- **Maintenance of the material as plantlets** and subsequent propagation from their nodal cuttings reduces the risk of genetic instability.

- A basic requirement for practical feasibility of a plant tissue culture method in germplasm conservation is to reduce the frequency of subcultures to the bare minimum. This can be mainly achieved by freeze-preservation, cold storage, low-pressure and low-oxygen storage.
• In vitro methods are the useful for conservation of vegetatively propagated plants, species with recalcitrant seeds and genetically engineered materials.

• The advantages of *in vitro* conservation are:
  – Requirement for little space for preservation of a large number of clonally multiplied plants.
  – Maintenance of the material in an environment free of pests.
  – Protection against natural environmental hazards.
  – Availability of nucleus stock to propagate a large number of plants rapidly whenever necessary.
  – Minimizing the obstacles generally imposed by quarantine systems on the moment of live plants across national boundaries since they are raised and maintained in an aseptic environment.
Three approaches for the in vitro conservation of germplasm

• Cryopreservation (freeze-preservation)

• Cold storage

• Low-pressure and low-oxygen storage
Cryopreservation

• Preservation in the frozen state.

• Brings the plant cell and tissue cultures to a zero metabolism or non-dividing state by reducing the temperature in the presence of cryoprotectants.
Cryopreservation

- Broadly means the storage of germplasm at very low temperatures:
  - Over solid carbon dioxide (at -79°C)
  - Low temperature deep freezers (at -80°C)
  - In vapour phase nitrogen (at -150°C)
  - In liquid nitrogen (at -196°C)
Cryopreservation

• Most commonly used cryopreservation is by employing **liquid nitrogen**. At the temperature of liquid nitrogen (-196°C), the cells stay in a completely inactive state and thus can be conserved for long periods.

• In fact, cryopreservation has been successfully applied for germplasm conservation of a wide range of plant species e.g. rice, wheat, peanut, cassava, sugarcane, strawberry, coconut. Several plants can be regenerated from cells, meristems and embryos stored in cryopreservation
Mechanism of Cryopreservation

• The technique of freeze preservation is based on the transfer of water present in the cells from a liquid to a 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 a standstill.
Precautions/Limitations for Successful Cryopreservation

• Good technical and theoretical knowledge of living plant cells and as well as cryopreservation technique are essential.

• Formation ice crystals inside the cells should be prevented as they cause injury to the organelles and the cell.

• High intracellular concentration of solutes may also damage cells.

• Sometimes, certain solutes from the cell may leak out during freezing.

• Cryoprotectants also affect the viability of cells.

• The physiological status of the plant material is also important.
Technique of Cryopreservation

- Development of sterile tissue cultures
- Addition of cryoprotectants and pretreatment
- Freezing
- Storage
- Thawing
- Re-culture
- Measurement of survival/viability
- Plant regeneration.
Development of sterile tissue culture

- The selection of plant species and the tissues with particular reference to the **morphological and physiological characters** largely influence the ability of the explant to survive in cryopreservation.

- **Any tissue from a plant can be used** for cryopreservation e.g. meristems, embryos, endosperms, ovules, seeds, cultured plant cells, protoplasts, calluses.

- Among these, **meristematic cells and suspension cell cultures**, in the late lag phase or log phase are most suitable.
Addition of cryoprotectants and pretreatment

- Cryoprotectants are the compounds that can **prevent the damage caused to cells by freezing or thawing**. The **freezing point and super-cooling point of water are reduced** by the presence of cryoprotectants. As a result, the ice crystal formation is retarded during the process of cryopreservation.

- There are several cryoprotectants which include dimethyl sulfoxide (DMSO), glycerol, ethylene, propylene, sucrose, mannose, glucose, proline and acetamide.

- Among these, **DMSO, sucrose and glycerol** are most widely used. Generally, a mixture of cryoprotectants instead of a single one is used for more effective cryopreservation without damage to cells/tissues.
Freezing

• The sensitivity of the cells to low temperature is variable and largely depends on the plant species.

• Four different types of freezing methods are used:
  – Slow-freezing method
  – Rapid freezing method
  – Stepwise freezing method
  – Dry freezing method
Slow-freezing method

• The tissue or the requisite plant material is slowly frozen at a slow cooling rates of 0.5-5°C/min from 0°C to -100°C, and then transferred to liquid nitrogen.

• The advantage of slow-freezing method is that some amount of water flows from the cells to the outside. This promotes extracellular ice formation rather than intracellular freezing. As a result of this, the plant cells are partially dehydrated and survive better.

• The slow-freezing procedure is successfully used for the cryopreservation of suspension cultures.
Rapid freezing method

• This technique is quite simple and involves plunging of the vial containing plant material into liquid nitrogen.

• During rapid freezing, a decrease in temperature -300° to -1000°C/min occurs. The freezing process is carried out so quickly that small ice crystals are formed within the cells.

• Further, the growth of intracellular ice crystals is also minimal. Rapid freezing technique is used for the cryopreservation of shoot tips and somatic embryos.
Stepwise freezing method

• This is a combination of slow and rapid freezing procedures (with the advantages of both), and is carried out in a stepwise manner.

• The plant material is first cooled to an intermediate temperature and maintained there for about 30 minutes and then rapidly cooled by plunging it into liquid nitrogen.

• Stepwise freezing method has been successfully used for cryopreservation of suspension cultures, shoot apices and buds.
Dry freezing method

• Some workers have reported that the non-germinated dry seeds can survive freezing at very low temperature in contrast to water-imbibing seeds which are susceptible to cryogenic injuries.

• In a similar fashion, dehydrated cells are found to have a better survival rate after cryopreservation.
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 temperatures in the range of -70 to -196°C.

• However, with temperatures above -130°C, ice crystal growth may occur inside the cells which reduces viability of cells. 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.

• A regular and constant supply of liquid nitrogen to the liquid nitrogen refrigerator is essential. It is necessary to check the viability of the germplasm periodically in some samples. Proper documentation of the germplasm storage has to be done.
i. Taxonomic classification of the material

ii. History of culture

iii. Morphogenic potential

iv. Genetic manipulations done

v. Somaclonal variations

vi. Culture medium

vii. Growth kinetics
Thawing

- Thawing is usually carried out by plunging the frozen samples in ampoules into a warm water (temperature 37-45°C) bath with vigorous swirling. By this approach, rapid thawing (at the rate of 500 - 750°C min⁻¹) occurs, and this protects the cells from the damaging effects of 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 (37-45°C) water bath.

- For the cryopreserved material (cells/tissues) where the water content has been reduced to an optimal level before freezing, the process of thawing becomes less critical.
Re-culture

- In general, thawed germplasm is **washed several times to remove cryoprotectants.**

- This material is then re-cultured in a fresh medium following standard procedures. Some workers prefer to directly culture the thawed material without washing. This is because certain vital substances, released from the cells during freezing, are believed to promote in vitro cultures.
Measurement of survival/viability

• The viability/survival of the frozen cells can be measured at any stage of cryopreservation or after thawing or re-culture.

• The techniques employed to determine viability of cryopreserved cells are the same as used for cell cultures. Staining techniques using triphenyl tetrazolium chloride (TTC), Evan’s blue and fluorescein diacetate (FDA) are commonly used.

• The best indicator to measure the viability of cryopreserved cells is their entry into cell division and regrowth in culture. This can be evaluated by the following expression.

\[
\frac{\text{No. of cells/organs growing}}{\text{No. of cells/organs thawed}} \times 100
\]
Plant regeneration

- The ultimate purpose of cryopreservation of germplasm is to regenerate the desired plant.

- For appropriate plant growth and regeneration, the cryopreserved cells/tissues have to be carefully nursed, and grown.

- Addition of certain growth promoting substances, besides maintenance of appropriate environmental conditions is often necessary for successful plant regeneration.
<table>
<thead>
<tr>
<th>Plant material</th>
<th>Plant species</th>
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<tbody>
<tr>
<td>Cell suspensions</td>
<td><em>Oryza sativa</em></td>
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<td></td>
<td><em>Glycine max</em></td>
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<td><em>Zea mays</em></td>
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<td><em>Nicotiana tabacum</em></td>
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<td><em>Capsicum annum</em></td>
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<tr>
<td>Callus</td>
<td><em>Oryza sativa</em></td>
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<td></td>
<td><em>Capsicum annum</em></td>
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<td></td>
<td><em>Saccharum sp</em></td>
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<td>Protoplast</td>
<td><em>Zea mays</em></td>
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<td></td>
<td><em>Nicotiana tabacum</em></td>
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<tr>
<td>Meristems</td>
<td><em>Solanum tuberosum</em></td>
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<td></td>
<td><em>Cicer arietinum</em></td>
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<tr>
<td>Zygotenic embryos</td>
<td><em>Zea mays</em></td>
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<td></td>
<td><em>Hordeum vulgare</em></td>
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<td><em>Manihot esculenta</em></td>
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<td>Somatic embryos</td>
<td><em>Citrus sinensis</em></td>
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<td><em>Daucus carota</em></td>
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<td><em>Coffea arabica</em></td>
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<td>Pollen embryos</td>
<td><em>Nicotiana tabacum</em></td>
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<td></td>
<td><em>Citrus sp</em></td>
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<td></td>
<td><em>Atropa belladona</em></td>
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</tbody>
</table>
Fig. 48.1: An outline of the protocol for cryopreservation of shoot tip (DMSO—Dimethyl sulfoxide).
Cold Storage

- Cold storage basically involves germplasm conservation at a low and non-freezing temperatures (1-9°C). The growth of the plant material is slowed down in cold storage in contrast to complete stoppage in cryopreservation. Hence, cold storage is regarded as a slow growth germplasm conservation method. The major advantage of this approach is that the plant material (cells/tissues) is not subjected to cryogenic injuries.

- **Long-term cold storage** is simple, cost-effective and yields germplasm with good survival rate. Many in vitro developed shoots/plants of fruit tree species have been successfully stored by this approach e.g. grape plants, strawberry plants.

- **Virus-free strawberry plants could be preserved at 10°C for about 6 years**, with the addition of a few drops of medium periodically (once in 2-3 months).

- **Several grape plants have been stored for over 15 years by cold storage (at around 9°C)** by transferring them yearly to a fresh medium.
Low-Pressure and Low-Oxygen Storage

- As alternatives to cryopreservation and cold storage, **low-pressure storage (LPS)** and **low-oxygen storage (LOS)** have been developed for germplasm conservation.
Low-Pressure Storage (LPS)

- In low-pressure storage, the **atmospheric pressure surrounding the plant material is reduced**. This results in a partial decrease of the pressure exerted by the gases around the germplasm. The lowered partial pressure reduces the in vitro growth of plants (of organized or unorganized tissues). Low-pressure storage systems are useful for short-term and long-term storage of plant materials.

- The short-term storage is particularly useful to increase the shelf life of many plant materials e.g. fruits, vegetables, cut flowers, plant cuttings. The germplasm grown in cultures can be stored for long term under low pressure. Besides germplasm preservation, LPS reduces the activity of pathogenic organisms and inhibits spore germination in the plant culture systems.
Low-Oxygen Storage (LOS)

• In the low-oxygen storage, the oxygen concentration is reduced, but the atmospheric pressure (260 mm Hg) is maintained by the addition of inert gases (particularly nitrogen).

• The partial pressure of oxygen below 50 mm Hg reduces plant tissue growth (organized or unorganized tissue).

• This is due to the fact that with reduced availability of $O_2$, the production of $CO_2$ is low. As a consequence, the photosynthetic activity is reduced, thereby inhibiting the plant tissue growth and dimension.
Limitations of LOS

• The long-term conservation of plant materials by low-oxygen storage is likely to inhibit the plant growth after certain dimensions.
Applications of Germplasm Storage

- Maintenance of stock cultures
- Cryopreservation is an ideal method for long term conservation of cell cultures which produce secondary metabolites (e.g. medicines).
- Disease (pathogen)-free plant materials can be frozen, and propagated whenever required.
- Recalcitrant seeds can be maintained for long.
- Conservation of somaclonal and gametoclonal variations in cultures.
- Plant materials from endangered species can be conserved.
- Conservation of pollen for enhancing longevity.
- Rare germplasms developed through somatic hybridization and other genetic manipulations can be stored.
- Cryopreservation is a good method for the selection of cold resistant mutant cell lines which could develop into frost resistant plants.
- Establishment of germplasm banks for exchange of information at the international level.
Limitations of Germplasm Storage

• The major limitations of germplasm storage are the expensive equipment and the trained personnel. It may, however, be possible in the near future to develop low cost technology for cryopreservation of plant materials.
National Seed Policy 1999 (2056 BS)

3.1 Varietal development and maintenance
3.2 Seed multiplication
3.3 Quality control
3.4 Increased involvement of private sector in seed business
3.5 Supply arrangements
3.6 Institutional strengthening
3.7 Bio/modern technology
Svalbard Global Seed Vault

- the capacity to store 1.5 million different seed samples
- samples of 526,000 unique crop varieties in storage (May 2010)
- storage in permafrost zone at -16 to -17 °C (today), at -18 °C (target temperature)
Cataloging

• Collected materials should be processed on site and immediately upon arrival to the genebank.

• Passport and associated information should be collated and documented.

• Field sampling (distribution of sites, number of sites, delineation of sites, distribution of plants sampled in sites, number of plants sampled)

• Population identification

• Collecting (passport data, Voucher specimens, germplasm, live plants)
Passport data

• Includes all basic information recorded during the time of collecting samples or the information provided by the sender regarding source/origin, etc.

• Useful for all phases of genetic resources work.

• Site identification is perhaps the most significant evidence available to the curator for designating a 'core collection' and is of great help in identifying duplicates.

• Essential information for eco-biological, evolutionary or population genetic research and for planning further collections.

• Records on topography or soil characteristics can be valuable for plant breeders too for improving adaptation to particular conditions or tolerance of edaphic or climatic stresses.
Important passport descriptors

- Site of collection (village/state/country);
- collector's number; type of material (population or pure line);
- date of collection;
- altitude, latitude and longitude for site of collection;
- status (wild, weedy, landrace, cultivar);
- growing conditions; and
- source (field, farm store, institute, etc.).
Evaluation

• Evaluation is usually done for traits such as **yield, agronomic performance, biotic and abiotic stress susceptibilities** as well as for biochemical and cytological traits.

• The expression of these traits is usually influenced by the environment and therefore may require special experimental designs and techniques.

• Evaluation is usually **done by a multidisciplinary team of scientists** which includes a breeder and other specialists (eg entomologist for insect resistance, physiologist for stress tolerance, pathologist for disease resistance)

• In practice, characterization and preliminary evaluation are usually **done during the initial seed increase or during the first regeneration cycle**
Methods of Evaluation

• The methods on the description of the germplasm include morphological and cytological, biochemical and molecular approaches.

• Morphological traits are evaluated based on the descriptor list.

• This can be supplemented by studying plants on the cellular level using cytology and cytogenetics.
Characterization and evaluation using morphological markers

• These are useful in the establishment of the chromosome number and genome composition of the genetic material.

• Another approach is the direct study of the genome using biochemical and molecular markers.

• Morphological characterization is the descriptor of an accession based on morphological markers evaluated at various stages of growth (seedling, vegetative, inflorescence, fruit and seed).

• Descriptors used in characterization of germplasm accessions may include morphological/botanical features, which may be mono/oligogenic, highly heritable and expressed within acceptable limits of deviation over a range of agroclimatic conditions (eg leaf shape, flower color, seedcoat color).
- Descriptor used in evaluation are generally useful for crop improvement.
- They are highly affected by the environment, involve complex biochemical or molecular processes and may include yield, agronomic performance, stress reactions etc.
Important characterization and preliminary evaluation descriptors and descriptor states

- **Site data:** Information on evaluation site (centre/institute, state, country), evaluator's name and evaluation date (month and year).
- **Planting data:** Propagation method (seed, cutting, grafts), habit, height, density of branches (sparse, dense), crown diameter, etc.
- **Leaf characters:** Type of leaf, petiole type, leaf size, leaflet type, etc.
- **Floral characters:** Arrangements of flowers, position of flowers, type of inflorescence, colour of flower bud, length of pedicel, length of bud, number of stamens, flower aroma, pollination, etc.
- **Fruiting characters:** Number of days from flowering to harvest, main harvest season, yield, etc.
- **Fruit characters:** Number of fruits/cluster, fruit length and width, protein (%), fat (%), shattering habit, seeds/fruit, etc.
- **Seed characters:** Seed size, hilum size and colour, 100-seed weight, etc.
Utilization of plant genetic resources.

• In order to fully utilize available genetic diversity in genebanks, prebreeding or parental line breeding of exotic/unadapted materials should be undertaken.

• Prebreeding/parental line breeding refers to all activities designed to identify desirable target characteristics and/or gene from unadapted (exotic or semi exotic) material, including those that, although adapted, have been subject to any kind of selection for improvement.

• Exotic materials include any germplasm that do not have immediate usefulness without selection for adaptation for a given area.

• Pre breeding/parental line breeding is a vital step to link conservation and use of plant genetic resources especially in breeding program.

• It aims at reducing genetic uniformity in crops through the introduction of a wider base of diversity as well as to increase yields, resistance to pests and diseases and other quality traits.
Prebreeding/parental line breeding programs can generate new base populations for breeding programs and also assist in identifying heterotic patterns for hybrid programs.

Prebreeding aims to provide breeders with enhanced germplasm materials which have specific traits of interest as well as a means to broaden the diversity of improved germplasm.
Consideration in parental line breeding

- Basic study of target characteristics
- Development of screening method
- Screen of germplasm for target characteristics
- Genetic variation and breeding population
- Screen of breeding population for target characteristics
- Identifying genes of target characteristics
- Release a new variety and use parental line with target characteristics