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Germplasm conservation, It's practices in Horticulture crops

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Germplasm conservation

Germplasm broadly refers to the hereditary material (total content of genes) transmitted to the offspring through germ cells. Germplasm provides the raw material for the breeder to develop various crops. Thus, conservation of germplasm assumes significance in all breeding programmes.

As the primitive man learnt about the utility of plants for food and shelter, he cultivated the habit of saving selected seeds or vegetative propagules from one season to the next one. In other words, this may be regarded as primitive but conventional germplasm preservation and management, which is highly valuable in breeding programmes.

The very objective of germplasm 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.

There are two approaches for germplasm conservation of plant genetic materials:

1. 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.

2. 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

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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.

Germplasm conservation in the form of seeds

Usually, seeds are the most common and convenient materials to conserve plant germplasm. This is because many plants are propagated through seeds, and seeds occupy relatively small space.

- Certain limitations in the conservation of seeds:
- Viability of seeds is reduced or lost with passage of time.
- Seeds are susceptible to insect or pathogen attack, often leading to their destruction.
- It is difficult to maintain clones through seed conservation.

In vitro methods for germplasm conservation

In vitro methods employing shoots, meristems and embryos are ideally suited for the conservation of germplasm of vegetatively propagated plants. The plants with recalcitrant seeds and genetically engineered materials can also be preserved by this in vitro approach.

There are several advantages associated with in vitro germplasm conservation:

- Large quantities of materials can be preserved in small space.
- The germplasm preserved can be maintained in an environment, free from pathogens.
- It can be protected against the nature's hazards.

There are mainly three approaches for the in vitro conservation of germplasm

1. Cryopreservation (freeze-preservation)
2. Cold storage
3. Low-pressure and low-oxygen storage

Cryopreservation

Broadly means the storage of germplasm at very low temperatures:

i. Over solid carbon dioxide (at -79°C)
ii. Low temperature deep freezers (at -80°C)
iii. In vapour phase nitrogen (at -150°C)
iv. In liquid nitrogen (at -196°C)

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.

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.

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.

Applications of germplasm storage

1. Maintenance of stock cultures: Plant materials (cell/tissue cultures) of several species can be cryopreserved and maintained for several years, and used as and when needed.
2. Cryopreservation is an ideal method for long term conservation of cell cultures which produce secondary metabolites (e.g. medicines).
3. Disease (pathogen)-free plant materials can be frozen, and propagated whenever required.
4. Recalcitrant seeds can be maintained for long.
5. Conservation of somaclonal and gametoclonal variations in cultures.
6. Plant materials from endangered species can be conserved.
7. Conservation of pollen for enhancing longevity.

Problems of recalcitrance

Recalcitrance poses a significant challenge to germplasm conservation, particularly in the preservation of certain plant species. Recalcitrant seeds and tissues are those that do not respond well to conventional storage methods and often lose viability or suffer damage during the conservation process.

Several factors contribute to recalcitrance, including high moisture content, desiccation sensitivity, and a susceptibility to chilling or freezing injury. These traits make it difficult to store these materials for extended periods, limiting their potential for use in future breeding programs or ecosystem restoration.

- Recalcitrance refers to inability of plant tissue culture to respond to culture manipulation. (loss of morphogenetic competence and totipotency capacity.)

- Free radicals have an important role in the metabolism and development of aerobic organisms
- Free radical-mediated stress has a role in tissue culture recalcitrance.
- In vitro plant systems have shown that tissue cultures produce free radicals, lipid peroxides and toxic, aldehyde lipid peroxidation products.
- Antioxidant protection is compromised oxidative stress ensues and free radicals and their reaction products react with macromolecules such as DNA, proteins and enzymes, causing cellular dysfunction and, as a result, the cultures become recalcitrant.

Addressing Recalcitrance Challenges

Researchers are actively working to overcome the challenges posed by recalcitrant germplasm, employing various strategies to improve storage and conservation techniques. Some of the key approaches include:

1. **Cryopreservation:** This involves ultra-low temperature storage of genetic material, often using liquid nitrogen. Cryopreservation has shown promise in maintaining the viability of recalcitrant seeds and tissues.
2. **In Vitro Culture:** Tissue culture methods, including somatic embryogenesis and organogenesis, can be employed to regenerate plants from small pieces of tissues, bypassing the challenges associated with recalcitrant seeds.
3. **Molecular Approaches:** Advances in molecular biology, such as the identification and manipulation of genes related to desiccation tolerance and storage protein synthesis, offer potential solutions to recalcitrance.

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