UNIT-2

Cultivation of Drugs

The crude drugs which reach the market and pharmaceutical industry will have passed from different stages that have some effect in the nature and amount of active constituents. Cultivation of Drugs is the first stage.

Advantages of cultivation

- ensures quality and purity of medicinal plants.
- better yield and therapeutic quality
- Regular supply of crude drugs
- industrialisation of medicinal and aromatic plants

Disadvantages

- losses occur due to natural factors, like floods, draught, snowfall, etc.
- cost of production is high for growing crude drugs.
- land, labour, money

Factors Affecting cultivation of Medicinal Plants

These are the following factors:

(i) Altitude, temperature and humidity
(ii) Rainfall or irrigation
(iii) Soil
(iv) Fertilizers and Manures
(v) Pests

1) Altitude, Temperature & Humidity

Altitude plays a crucial role. For example, tea is grown at an altitude of 1000-1500 metres, cinchona at 1000-2000 metres, cinnamon at 250-1000 metres, clove at upto 900 metres. This altitude is measured as height from sea level.

Temperature also plays crucial role. Extremely high as well as low temperature disturbs the quality of medicinal plants. For example, camphor and coffee cannot grow favourably at extremely low temperatures; saffron grows in cold climate and pyrethrum requires a dry weather.

And if the humidity is extremely high or low, the plant growth is not favourable.

2) Rainfall or Irrigation

The xerophytic plants, e.g., aloe, acacia, etc. can be grown even if irrigation is not adequate. otherwise, a suitable irrigation arrangement or sufficient rainfall is required for optimum growth. Some plants even require high amount of rainfall.

3) Soil

Soil is the natural resource required for plant growth as it provides mechanical anchorage, water, essential food elements. Soil should be rich in nutrients, organic matter and other elements. Following soil conditions should be maintained as per the selected medicinal plants:

(i) Soil type → Soil is considered to be poor if it contains organic matter less than 0.5% and rich in organic matter content if it is more than 1.5–5%.

(ii) Soil pH → The soil having pH 6.5 to 7.5 contains maximum amount of plant nutrients. The alkaline soil pH can be neutralised by liming the acidic soils and gypsum

(iii) Soil fertility → Soil provides plants with natural nutrients.

4) Fertilisers and Manures

Soil provides nutrients to plants in sufficient amounts. But soil loses its fertility if cropping is done without nurturing it with nutrients. Leaching and erosion also affect soil fertility. Animal manures, nitrogen-fixing bacteria, or chemical fertilisers should be added to soil to maintain fertility.

Three types of fertilisers are used:

(i) Chemical fertilisers (synthetically prepared)

(ii) Biofertilisers (symbiosis, nitrogen-fixing bacteria)

(iii) Manures (Farmyard manure/compost)

5) **Pests**

Undesired animal species is termed pest. chemicals used against them are pesticides.

Types of pests

(i) Fungi (Ascochyta atropae causes leaf necrosis)
(ii) Viruses (Tobacco Mosaic Virus)
(iii) Insects (caterpillar)
(iv) Weeds (these also come under pests - datura)
(v) Non-insect pests → rats, birds

Pest Management

Control of pests is termed pest management.
Pest management can be done by following:

1) **Mechanical Methods** → This involves collection and destruction of pests using different devices. Hand-picking, pruning, burning, pests trapping are some simple methods.

2) **Agricultural Methods** → This involves producing pest-resistant plant species by genetic manipulations. Hybrid varieties resistant to fungal and bacterial attack have also been produced by this technique. Ex- Bt cotton, Bt Brinjal

3) **Biological Methods**
This method involves combating pests with other living organisms. This method is effective, safe and economic.
Examples- birds eat insect pests, rat terriers eat rats.

4) **Chemical Methods** → involves use of chemical pesticides to control pests.
Some pesticides are:
(i) Rodenticides (warfarin, strychnine)
(ii) Insecticides (D.D.T, gammaxine)
(iii) Acaricides - against mites and ticks e.g. chlorobenzolate, tetradifon
(iv) Fungicides (chlorophenols, antibiotics)
(v) Herbicides (calcium arsenate, H_2SO_4)

Methods of Propagation of Crude Drugs

Plant propagation is the process by which new plants grow from various sources including seeds, cuttings, and other plant parts.

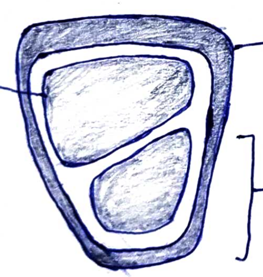
There are two methods of propagation:

(i) Sexual Method (seed propagation)

(ii) Asexual method (vegetative propagation)

Sexual Method

Sexual propagation involves the fusion of pollen with egg to produce a seed. The seed is made up of three parts: the outer seed coat, which protects the seed; the endosperm, which is a food reserve; and the embroy, which is the young plant itself.



A seed diagram

Small plants obtained from are called seedlings.

Some of the methods which employ seeds for cultivation:

(i) Broadcasting method

The seeds are scattered in well-prepared soil. They may fail to germinate if sown deeply.
examples- linseed and sesame seeds.

(ii) Dibbling → This method is applied when seeds are of average size or weight.
They are placed in holes made in the soil.
Example- Funnel (4-5 fruits are sown in each hole, castor (2-3 seeds are placed)

Advantages of sexual Propagation

(i) easiest and least expensive

(ii) Seedling plants are long-lived, productive and have greater tolerance to adversity.

(iii) Propagation of plants like papaya, phalsa and coconut is feasible in which asexual means of propagation is not common.

(iv) Hybrids can only be developed by sexual means.

Disadvantages of sexual method

(i) low yield in comparison to grafted trees.
(ii) take much longer to bear fruits
(iii) require more spraying of pesticides.

Vegetative Method

In the asexual method, its vegetative part (stem or root) is placed under favourable conditions to make it develop into a new plant.

Methods of vegetative propagation:

1) **Natural Methods** → These methods involve sowing various plant-parts in well-prepared soil.

Runners or stolons → Stems that grow horizontally on ground.
e.g. Mint, strawberry.

Bulb → Bulbs have a bud surrounded by layers of fleshy leaves.
e.g. onions, garlic, tulips.

Tubers → are fleshy underground storage structures.
e.g. potatoes, aconite.

offsets → run on the ground with a tuft of leaves and a cluster of roots below.

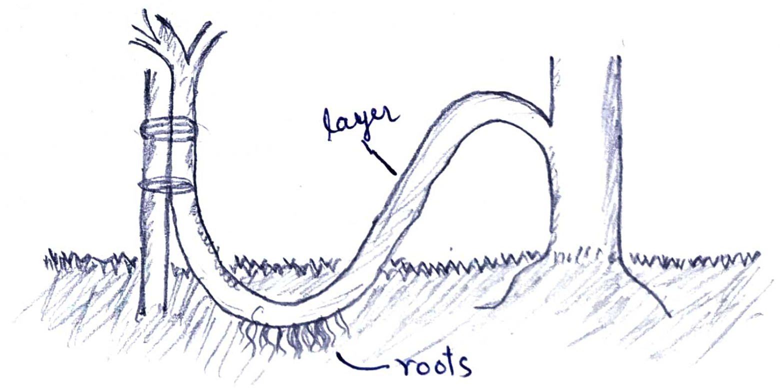
rhizomes → are root-like stems that grow horizontally under the ground.
e.g. ginger, turmeric

2) **Artificial Methods** → involves the production of seedlings from the vegetative part.

This is done by following methods:

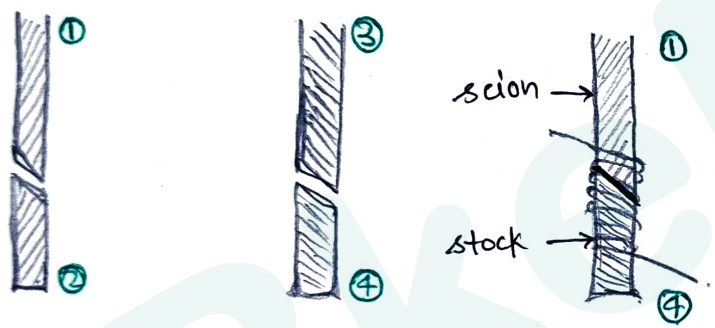
(i) **Cuttings** (a cutting is buried in the soil)
- stem-cuttings (orange, rose, berberry)
- root cuttings (brahmi)
- leaf cuttings (bryophyllum)

(ii) **Layering** - a branch of the plant is covered by soil. Once the roots grows, the new plant is detached from the parent.



Some of the plants in which layering is used → guava, lemon, jasmine, mango, etc.

(iii) Grafting → It is a method in which the stem of two plants are cut. These stems are then joined, one with root and another without root.



3) Tissue Culture

The plant parts such as (single cells, callus, seeds, embryos, root tips, shoot tips, etc.) are developed in artificial medium and providing them with nutritional and hormonal requirements.

Advantages of Vegetative Propagation

(i) The plant grown is similar to its plant source.
(ii) The seedless varieties of fruits can be propagated
(iii) Fruiting starts much earlier than seedlings.
(iv) Disease resistant plants by budding or grafting.

Disadvantages

(i) Such plants do not grow large in size.
(ii) not long lived
(iii) No new varieties of plants can be produced.

Collection and Processing of Drugs

Medicinal plant materials should be collected during the appropriate season or time period to ensure the best possible quality of products.
After collection of crude drugs, they are processed for marketing.
The drugs should be preserved in well-closed or filled containers and stored in water-proof, fire-proof, and rodent-proof places

Time of collection

Crude drugs should be collected when the medicinal activity is highest of the plant

Example- stromonium leaves are gathered in the morning. In the morning, they contain the higher proportion of alkaloids than in the evening.

Collection of Different Parts of Plants

1) Leaf and flowering tops → these are collected before they get mature (e.g. senna, digitalis, vinca) while aloe leaves are collected when they become adequately thick.

2) Barks → These are collected in spring and early summer when cambium is active. wild cherry is collected in autumn and cinnamon in the rainy season

3) Fruits → These are collected either ripe or half-ripe but fully grown.

Roots & Rhizomes → Roots are collected in spring. Rhizomes are collected when they have a rich amount of food material and chemical constituents.

Resins, Gums & Lattices → These unorganised drugs are collected when they start oozing out of the plants.

Harvesting

During harvesting, Pharmacopoeial standards should be followed.

An efficient harvesting is done by skilled workers only.

Various techniques of Harvesting

1) Binders → These are used for harvesting drugs which constitute all aerial parts.

2) Seed stripper → This device is used for harvesting flowers, seeds and small fruits.

3) Beating with bamboo → used for harvesting cloves.
4) Brushing → used for collecting cochineal insects from branches of cacti.
5) Handled Forks → used for harvesting seaweeds.
6) Reaping Machines → Used for harvesting fennel, coriander.

Drying

Drying involves removal of sufficient moisture from the crude drug for obtaining a good quality finished product. Drying helps in resistance to microbial growth and inhibits enzymatic reactions. Drying also eases the grinding of a crude drug.

Types of drying

1) Natural Drying → It is sun-drying or in shed.
Drying in sunlight is preferred if the drug contents are stable to high temperature and sunlight (e.g. gum acacia, seeds and fruits).
Drying in shed is preferred if the natural colour of the drug (digitalis, clove, senna) and its volatile ingredients (peppermint) are to be retained.

2) Artificial Drying
Hot air of desired temperature is circulated through the drugs.
Examples - belladona roots, cinchona bark, tea and raspberry leaves and gums are dried by this method.

Garbling

After drying the crude drug preparation, garbling is carried out to remove sand, dirt and foreign organic parts of the same plant from the drug.

Packing

During packaging of drugs, their morphological and chemical nature, uses and effects of climatic conditions during transportation and storage should be considered.

Storage

(i) Storage facilities for medicinal material should be well aerated, dry and protected from sunlight and humidity.

(ii) The floor should be tidy, without cracks and easy to clean. Medicinal material should be stored in shelves and at a sufficient distance from the walls.

(iii) Continuous in-process quality control measures should be implemented to eliminate substandard materials, contaminants and foreign matter.

(iv) Materials used for packaging should be non-polluting, clean, dry and in undamaged condition.

(v) Fresh medicinal plant materials should be stored at appropriate low temperatures ideally at 2-8°C.

(vi) Wooden boxes and paper bags should not be used for storage of crude drugs.

(vii) Dried medicinal plants including essential oils, should be stored in a dry, well-aerated building.

Plant Hormones and Their Applications

Naturally occurring growth regulators which are required in low concentrations for controlling physiological processes in plants are called plant hormones or phytohormones. They may be natural or synthetic. The synthetic growth regulators are structurally and functionally similar to natural growth hormones.

Naturally produced plant growth regulators are of 5 types.

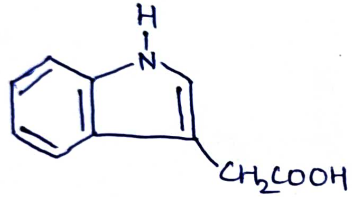
(i) Auxins
(ii) Gibberellins
(iii) Cytokinins
(iv) Ethylene
(v) Abscisic Acid

Auxins

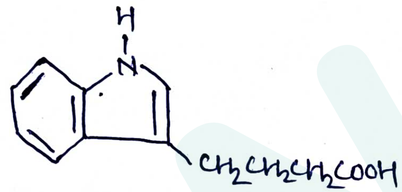
Auxins are growth promoters produced by growing apex of stem and roots. Thus, they promote elongation of shoot and root tips behind the apical meristem.

Auxins are of 2 types:

(i) Natural Auxins → These are produced by the plants. For example- Indole Acetic Acid (IAA) is the chief auxin. others are Indole-3-acetonitrile (IAN), Phenyl acetic acid.



IAA - natural



IBA - synthetic

(ii) Synthetic Auxins → similar to natural auxins but are not produced by plants.
e.g. Indole-3-butyric acid (IBA), α-Naphthyl acetic acid (NAA).

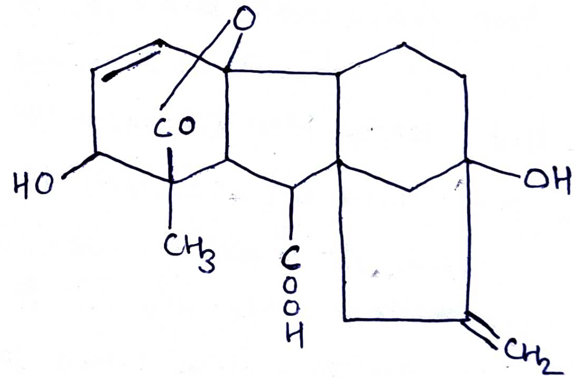
Functions

(i) They promote cell elongation.
(ii) promote cell division at root and shoot apex.
(iii) They promote leaf abscission, i.e., delays the shedding of leaves, flowers or fruits
(iv) induce parthenocarpy, i.e., development of seedless fruits without pollination or fertilisation.
(v) They differentiate the xylem and phloem.

Gibberellins

They are produced in the plant embryos, roots, and young leaves and promote growth. Roots, shoots, buds, leaves, floral apices, root nodules, fruits and callus tissues are the sites where gibberellins are present.

Gibberellin A is formed by a mixture of 6 gibberellins (GA_1, GA_2, GA_3, GA_4, GA_7 and GA_9). GA_3 is termed as gibberellic acid.



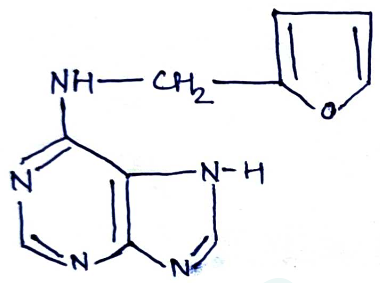
gibberellic acid

Functions

(i) promote stem elongation in genetically dwarfs.
(ii) break dormancy of seeds and buds.
(iii) induce parthenocarpy
(iv) They cause maleness in plants, thus help in sex expression.

Cytokinins

Cytokinins are either natural (zeatin) or synthetic (kinetin) compounds which regulate plant growth. They are produced in root apex, seed endosperm and in young fruits where the cells constantly divide.



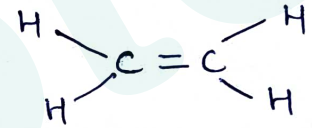
Kinetin

Functions

(i) They stimulate cell division, cell enlargement and cell differentiation.
(ii) prevent ageing of plant parts
(iii) They suppress apical dominance, i.e., allows the lateral buds to branch.
(iv) They induce femaleness in plants.
(v) regulate morphogenesis, i.e., initiation of organs.
(vi) help in breaking dormancy of many seeds, allowing them to germinate.
(vii) Induce parthenocarpy

Ethylene

Ethylene is an organic molecule which exists in the form of volatile gas. It is present in ripen fruits, flowers, seeds, stems, roots and tubers. It is also known as stress hormone.



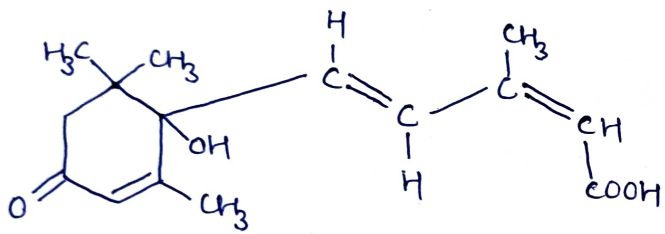
ethylene

Functions

1) It is a ripening agent, thus promotes fruits ripening.
(ii) It promotes senescence and abscission of leaves and flowers.
(iii) It increases cell width, but not length.
(iv) It increases the number of female flowers, reducing the male one.
(v) It inhibits stem elongation, causes swelling of nodes and nullifies geotropism.
(vi) Although it delays flowering in plants but in pineapples, it induces flowering.

Abscisic Acid

Abscisic acid is a growth inhibitor. It is produced in the leaves and carries out various physiological activities in plants such as retaining or shedding of leaves, stems, flowers and fruits.



Abscisic Acid

Functions

1) It causes dormancy of buds and seeds.
2) promotes leaf senescence, i.e., shedding of leaves.
3) It inhibits germination and development of seeds.
4) It reduces transpiration rate by promoting closing of stomata.
5) It delays flowering of long day plants.
6) It induces formation of tubers in potato.
7) It delays cell division and cell elongation.

Polyploidy

Generally, plants are diploids, means, they contain two sets of chromosomes, derived at fertilisation. One set of chromosomes come from pollen and another from the egg cells and denoted by '2n'.

The term 'polyploidy' is applied to plants with more than two sets of chromosomes.

A stage of 'haploidy' may also occur in some plants where they only have one set of chromosomes.

Types

1) Haploid (1n);
2) Diploid (2n)
3) Triploid (3n); Tardigrada
4) Tetraploid (4n); salmonidae fish
5) Pentaploid (5n); Kenai birch
6) Hexaploid (6n); wheat and Kiwifruit
7) Octaploid (8n); Acipenser
8) Decaploid (10n); certain strawberries
9) Dodecaploid (12n); Celosia argentea

Causes or Origin of Polyploidy

(i) Chromosome doubling during the early stage of embryo development.

(ii) One or both gametes may be unreduced (i.e., gametes with somatic chromosome number) in chromosome number due to some reason.

(iii) Fusion of two male gametes with a single egg cell gamete forming a triploid.

Techniques of Inducing Polyploidy

Polyploidy can be induced by methods following:

1) **Decapitation** → It the tip of certain seedlings is removed or cut by a sharp knife, callus is produced forming some polyploids.

2) **Radiations** → On exposing the vegetative and flower buds to X-rays, γ-rays or UV rays, polyploids are formed in some frequencies.

3) **Temperature** → On applying heat and cold shocks to flowers at or near the time of first zygote division forms polyploids.

4) **Hybridisation** → It also forms polyploids.

5) **Chemicals** → Chloral hydrate, acenaphthalene, coumarine, vertanine sulphate, colchicine, etc. are chemicals forming polyploids. Colchicine gives most effective results, thus widely used on all plant species.

Aneuploidy

Aneuploidy → Euploidy is a condition in which an individual has the perfect number of chromosomes (what we have discussed so far). While, aneuploidy is the condition in which an individual plant has one or more chromosomes added or deleted to the normal chromosome number.

One chromosome added condition – (2n+1)

One chromosome deleted → (2n−1)

Applications of Polyploidy

(i) In *Solanum khasianum*, it increases the solasodine content by 35–50%.

(ii) Increases in plant height and total fruit yield.

(iii) Increase in root weight and total alkaloids in leaves and roots in *Catharanthus roseus*.

(iv) Larger stomata, broader leaves, slower growth rate and higher pollen sterility.

(v) Increase in thebaine content in *Papaver bracteatum*.

Mutation

Hugo De Vries coined the term mutation, which means sudden change in the gene structure. The changes may arise spontaneously or when exposed to UV radiations or chemicals. The agents which cause mutations are called mutagens, and the cell or organism which shows the effect of mutations are called mutant.

Mutations can have the following features:

(i) It can occur at any stage of plant development.

(ii) It occurs either spontaneously or artificially induced.

(iii) It can prove to be harful as well as beneficial.

(iv) It can occur in all living plants.

Types of mutations in Medicinal Plants

1) Chromosomal mutation

It causes change in amount or position of genetic material.

2) Point Mutation → It is permanent and inheritable. It causes change in gene or cistron of DNA molecule.

3) Spontaneous mutation → It arises through unknown natural cause.

4) Induced Mutation → It is artificially induced by mutagens.
(e.g. exposure to UV rays, X-rays, ioning radiations, some chemicals, etc.)

5) Chemical Mutation

chemical mutagens (e.g. nitrogen mustard, formaldehydes) bring about chemical changes in the constitution of DNA bases.

Applications of Mutations

(i) aids in development of cultivation technology.

(ii) chemical mutagens raise the yield of morphine in *Papaver somniferum*.

(iii) Radiations increase the tuber yield and diosgenin content in Dioscorea bulbifera.

(iv) The contents of capsaicin in Capsicum annuum are elevated.

Hybridisation

It is mating or crossing of two genetically dissimilar plants having desired genes.

Two different plants having the desired characteristics are crossed together genetically to form one indidividual plant called hybrid.

Genetic contents of plants are not altered after hybridisation, in fact, a new combination of genes is produced.

Objectives of hybridisation

(i) Artificially creating a population with the desired combination of characters can be selected.

(ii) Combining the desired characters in a single individual.

(iii) Exploiting and using the varieties of hybrid.

Types of Hybridisation

1) Intra-Varietal → hybridization between the plants of same variety.

2) Inter-varietal → crossing between the plants of two different varieties of same species.

3) Interspecific → crossing between two different species of same genus.

4) Introgressive → certain genes of one species are transferred to the genome of other species.

Procedure of Hybridization

step-by-step process of hybridization below:

1) Selection of parents → the individual plants to be crossed are selected. At least one should be well-adopted and proven variety of the local area and the other variety should have the characters that are absent in the first chosen variety.

2) **Artificial Self-Pollination** → It induces homozygosity essentially to eliminate the undesirable characters and to get inbreeds.

3) **Emasculation** → Removal of stamens or anthers of a flower without affecting the female reproductive organs. Emasculation is essential in bisexual flowers. This is done to prevent self-pollination.

4) **Bagging** → Immediately after emasculation, the flowers or inflorescences are enclosed in bags of suitable sizes to prevent pollination by any foreign pollen.

5) **Crossing** → In this step, the fertile and viable pollens obtained from male parent are located on receptive stigma of emasculated flowers to carry out fertilisation.

6) **Raising F_1 Plants** → Pollination is naturally followed by fertilisation. It results in the formation of seeds. Mature seeds of F_1 generation are harvested, dried and stored. These seeds produce F_1 hybrids.

Applications in Medicinal Plants

(I) Cinchona succirubra and Cinchona ledgeriana are crossed to produced an increased amount of quinine.

(II) Withania somnifera (Israeli) and Withania somnifera (South African) are crossed to produce new hybrids with desired characters.

Conservation of Medicinal Plants

The traditional healthcare practice is mainly dependent on medicinal plants collected from the wild. With this, the medicinal plant biodiversity is being depleted due to man-made and natural calamities. Moreover, the indigenous (local) knowledge associated with the conservation and use of medicinal plants is also disappearing at an alarming rate. The fact that medicinal plants could be used as sources of revenue for farmers, the Institute of Biodiversity conservation (IBC) has initiated the development of a project on Conservation and Sustainable Use of Medicinal Plants (CSMPP).

Need for conservation

The goal aligns with the sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species.

Generally, there are two methods for conservation:

(i) In-situ (on-site) → conservation in the local habitat of the species.

(ii) Ex-situ (outside the native habitat)
It protects the populations at risk of destruction, replacement or deterioration.

Germplasm Technique for Conservation

The hereditary material (total content of genes) transmitted to the offring through germ cells is termed germplasm.

Germplasm conservation aims at preserving the genetic diversity of a particular plant for its further use.

Disadvantages of Germplasm

(i) Some crops do not produce viable seeds
(ii) Some seeds remain viable for a limited duration only.
(iii) Seeds of certain species deteriorate rapidly due to seed-borne pathogen.

Germplasm conservation may be done:
- in-situ
- ex-situ

Germplasm Conservation in the Form of Seeds

This is easiest and widely accepted method due to following reasons:
- occupy a much smaller space
- can be easily transported
- many plants are propagated using seeds

However, there are certain limitations also:
- seeds lose viability with time
- Seeds are more prone to insect or pathogen attack
- This approach is limited only to seed propagating plants.
- Maintaining clones through seed conservation is not possible.

In Vitro Conservation of Germplasm

In-vitro methods utilises shoots, meristems and embryos. Thus, they are suitable for vegetatively propagated plants.

Advantages

(I) Materials in large quantities can be preserved in small space.

(II) Large number of plants can be obtained from the germplasm stock.

In-vitro conservation of germplasm can be carried out by following approaches;

(i) Cryopreservation

(ii) Cold storage

(iii) Low-pressure and low-oxygen storage

Cryopreservation → preservation in the frozen state. The most common cryoprotectant used in cryopreservation technique is liquid nitrogen.

Cold-storage → conservation of germplasm at low but non-freezing temperatures ranging from 1–9°C.

Low-pressure and Low-oxygen

Low pressure storage (LPS) and Low oxygen storage (LOS) are the alternatives to cold storage and cryopreservation.

LPS

The surrounding atmospheric pressure of the plant material is lowered which reduces the in-vitro growth of plants.

LOS

Oxygen level around the plant material is lowered which reduces the plant growth because in absence or low oxygen, less carbon dioxide is produced.