IIIrd BSC BOTANY

18BBO66S - SKILL BASED SUBJECT – IV PLANT TISSUE CULTURE

Unit –V

Production of secondary metabolites – alkaloids, steroids, and terpenoids (Brief account only). Cryopreservation and germplasm preservation.

What are secondary metabolites?

- Secondary metabolites are generally defined as small organic molecules produced by an organism that are not essential for their growth, development and reproduction.

- They may include pharmaceuticals, flavours, fragrance, food additives, feedstock etc.

Why plant produce secondary metabolites?

- Plant hormones, which are secondary metabolites, are often used to regulate the metabolic activity within cells and oversee the overall development of the plant

- It protect plant against herbivores and microbial pathogens.

- It serves as attractants for pollination and seed dispersing animals.

Type of secondary metabolites

Type	Example	Uses
Alkaloids	Caffeine, Codeine, Quinine	Stimulant, Analgesic, Antimalarial
Cyanogenic glycoside	Diosgenin	Progesterone
Flavonoids	Quercetin, Procyanidins	Antibacterial, Antioxident, Anti-inflammatory
Phytic acid	-	Antioxident
Gossypol	Hypokalemic paralysis	-
Phytoestrogens	Resveratrol	Reduce risk of cardiovascular disease
Carotenoids	α-carotene, β-carotene and lycopene	Contribute to photosynthesis

Hormone	Applications
Abscisic acid	Inhibit growth in response to change in temperature and light. Control closing of stomata in dry condition. ABA is applied on plants beefore shipping
Auxins	Stimulate cell elongated, differentiation of xylem and phloem, root initiation. Suppress growth of lateral buds Delay leaf senescence
Cytokinins	Promotes cell division Lateral bud development Delay of senescence
Ethylene	Fruit ripening Inhibition of stem elongation
Gibberellins	Synthesis in apical portions of stems and roots. Important effects on stem elongation

Why in vivo production?

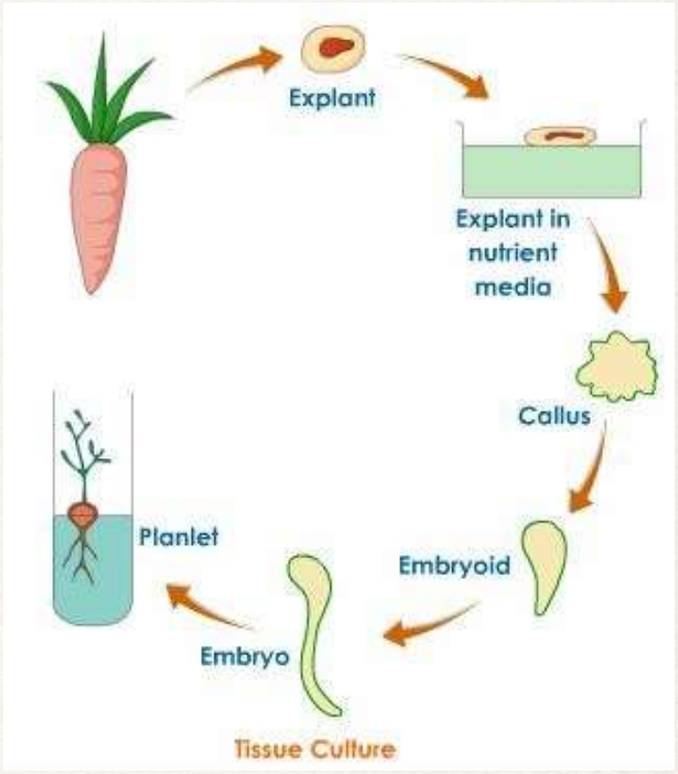
- According to WHO survey approximately 70-80% of world's total population depends on herbal drugs.

- Some compounds are difficult to synthesise chemically due to their structural complexity.

- Some novel compounds produced in cell cultures are not produced in intact plants. At least 85 novel compounds including 23 alkaloids, 19 terpenoids, 30 quinones and 11 phenyl compounds have been isolated from some 30 different plant culture systems.

Methods of in vivo culturing

- Callus Culture
- Suspension culture



Process of suspension Culture

1) Callus culture

- The first step to establish cell suspension cultures is to raise callus from any explants of the plant. To maximise the production of a particular compound, it is desirable to initiate the callus from the plant part that is known to be high producer.

- Calli are generally grown on medium solidified with gelling agent.

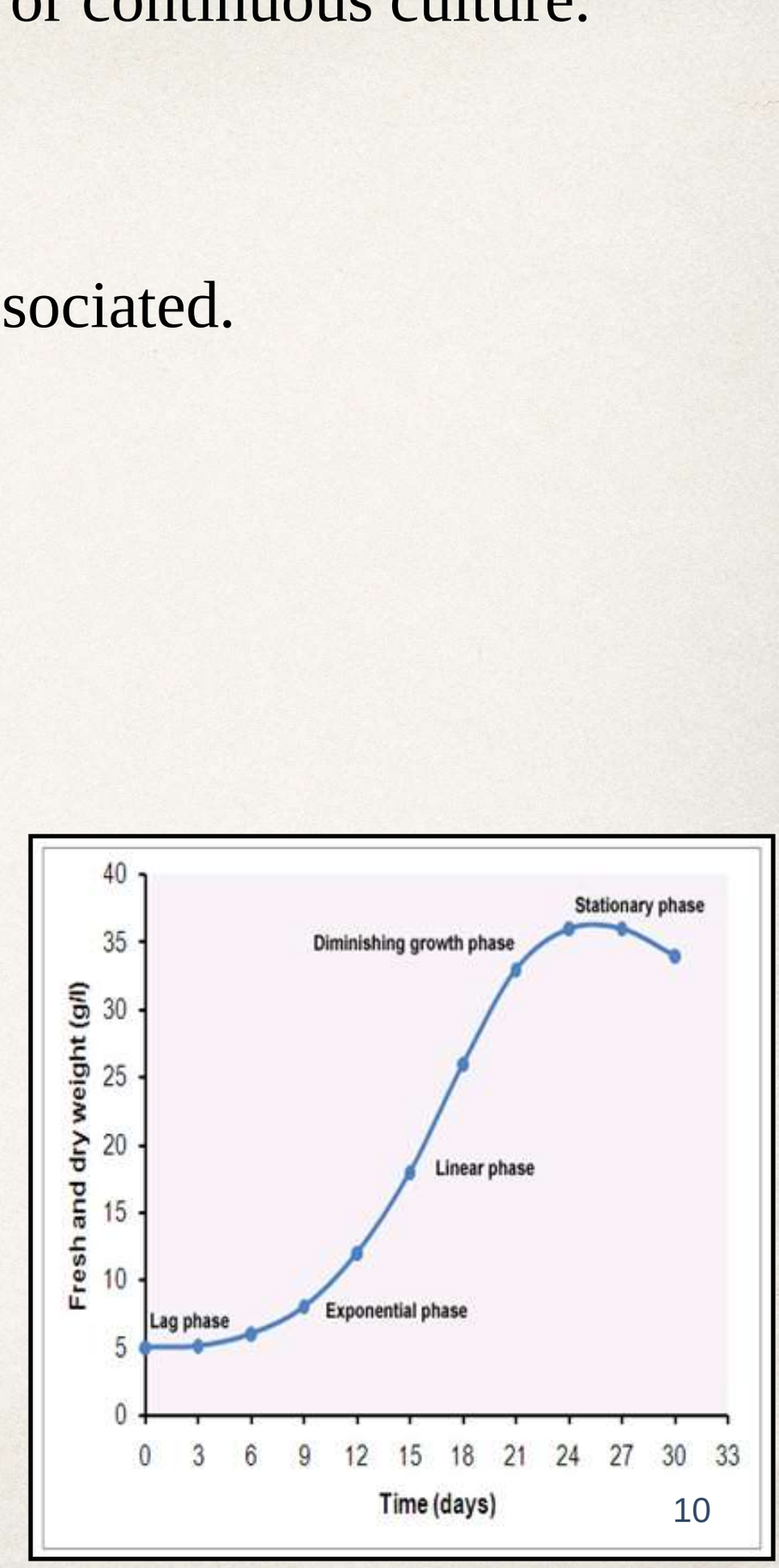
- In morphological terms it can vary extensively, ranging from being very hard/compact and green or light green in color, where the cells have extensive and strong cell to cell contact. and friable where the callus consists of small, disintegrating aggregates of poorly-associated cells and has brownish or creamy appearance.

List of products prepared by different culture methods

Plant name	Active ingredients	Culture type	References
Ailanthus altissima	Alkaloids	Suspension	Anderson *et al.* 1987[78]
A. altissima	Canthinone alkaloids	Suspension	Anderson *et al.* 1987[79]
Allium sativum L.	Allicin	Callus	Malpathak and David, 1986[80]
Aloe saponaria	Tetrahydroanthracene glucosides	Suspension	Yagi *et al.* 1983[81]
Ambrosia tenuifolia	Altamisine	Callus	Goleniowski and Trippi, 1999[82]
Bupleurum falcatum	Saikosaponins	Callus	Wang and Huang, 1982 [83]
Cassia acutifolia	Anthraquinone	Suspension	Nazif *et al.* 2000[83]
Catharanthus roseus	Indole alkaloids	Suspension	Moreno *et al.* 1993[84]
C. roseus	Catharanthine	Suspension	Zhao *et al.* 2001[85]
Citrus sp.	Narigin, Limonin	Callus	Barthe *et al.*1987[86]
Coffea arabica L.	Caffeine	Callus	Waller *et al.* 1983[87]
Corydalis ophiocarpa	Isoquiniline alkaloids	Callus	Iwasa and Takao, 1982[88]
Croton sublyratus kurz	Plaunotol	Callus	Morimoto and Murai, 1989[89]
Cruciata glabra	Anthraquinones	Suspension	Dorenberg and Knorr, 1996[90]
Dioscorea doryophora Hance	Diosgenin	suspension	Huang *et al.* 1993[91]
Ephedra spp.	L-ephedrine, D- pseudoephedrine	suspension	O'Dowd *et al.* 1993[92]
Fumaria capreolata	Isoquinoline alkaloids	suspension	Tanahashi and Zenk, 1985[93]
Eucalyptus tereticornis SM.	Sterols and phenolic compounds	callus	Venkateswara *et al.* 1986 [94]
Ginkgo biloba	Ginkgolide A	suspension	Carrier *et al.* 1991[95]
Glycirrhiza glabra var.glandulifera	Triterpenes	callus	*Ayabe et al.* 1990[96]
Isoplexis isabellina	Anthraquinones	suspension	Arrebola *et al.*1999[97]
Linum flavum L.	5-Methoxypodophyllotoxin	suspension	Uden *et al.* 1990[98]
Mucuna pruriens	L-DOPA	Suspension	Wichers *et al.* 1993[99]
Nothapodytes foetida	Camptothecin	Callus	Thengane *et al.* 2003[100]
Panax notoginseng	Ginsenosides	Suspension	Zhong and Zhu, 1995[101]
Ruta sp.	Acridone and furoquinoline	Callus	Baumert *et al.* 1992[102]

- Suspension culture could be run as batch culture or continuous culture.
- Predominantly batch culture is used because of:
  - Many secondary products are not growth associated.
  - Genetic instability of cultured cells.
  - Operability and reliability.
  - Economic considerations.

The growth of a cell suspension culture with respect to time is best described by the sigmoid curve. The growth rate is measured by the steepness of the curve, and it is the steepest when the population density reaches one-half of the carrying capacity.

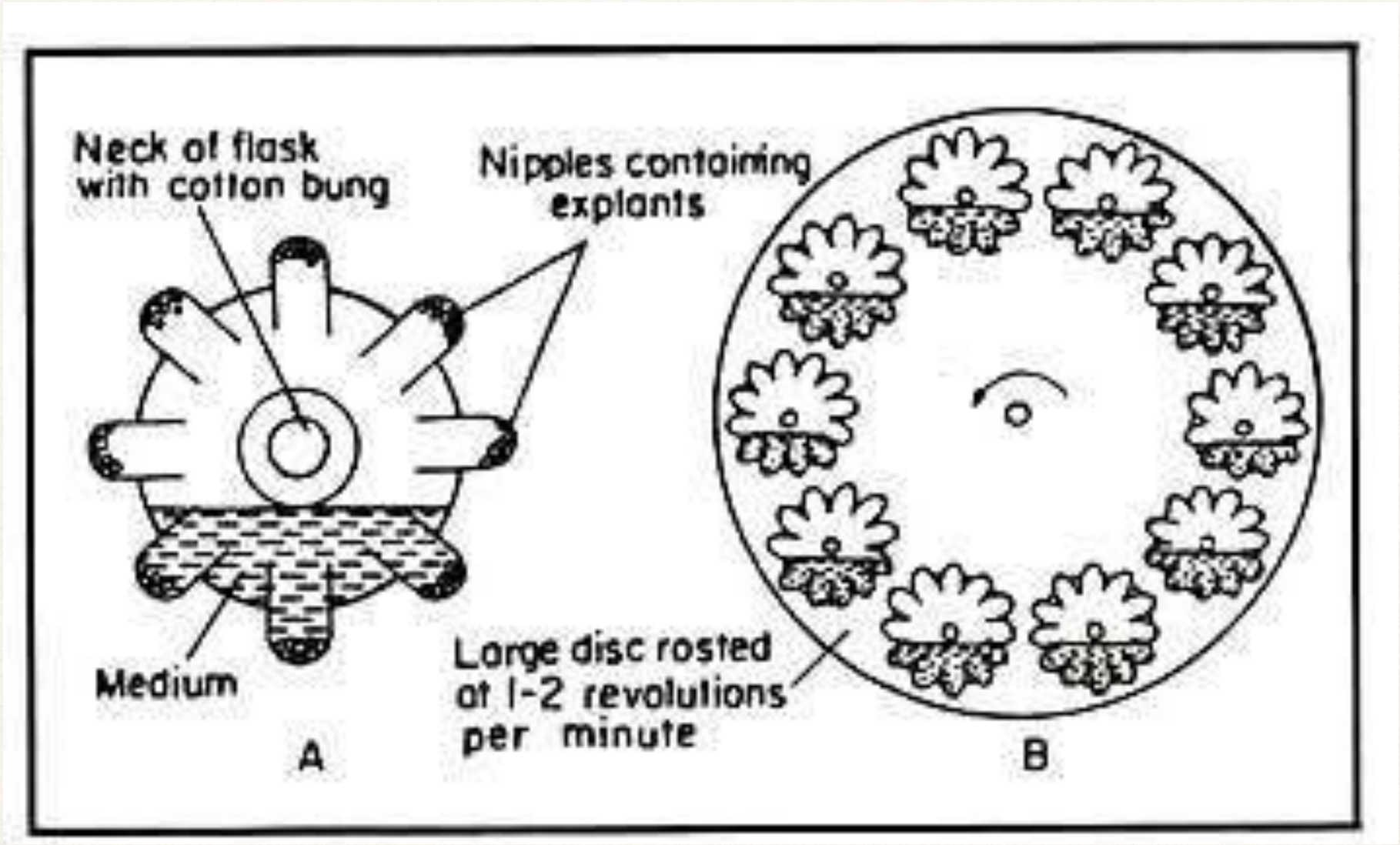


Types of Suspension culture

- **Batch culture**
  - Slowly rotating culture
  - Shake culture
  - Spinning culture
  - Stirred culture
- **Continuous culture**
  - Chemostats
  - Turbidostats

Slow motion rotating flask

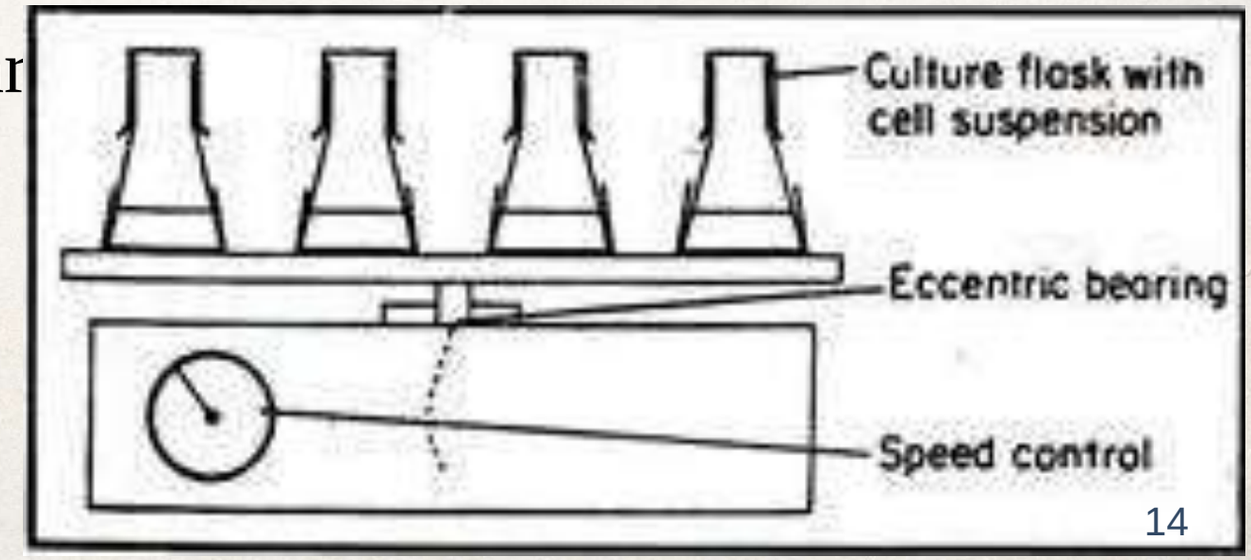
- In this culture, single cells and cell aggregates are grown in a specially designed flask, the nipple flask.
- Each nipple flask possesses eight nipple like projections, having a capacity of 250ml.
- They are loaded in a circular manner on the large flat disc of vertical shaker.
- When the flat disc rotates at a speed of 1-2rpm, the cells within each nipple of the flask are alternatively bathed in the culture medium and


Neck of flask
with cotton bung
Nipples containing
explants
Medium
A
Large disc rosted
at 1-2 revolutions
per minute
B

Shaker Culture

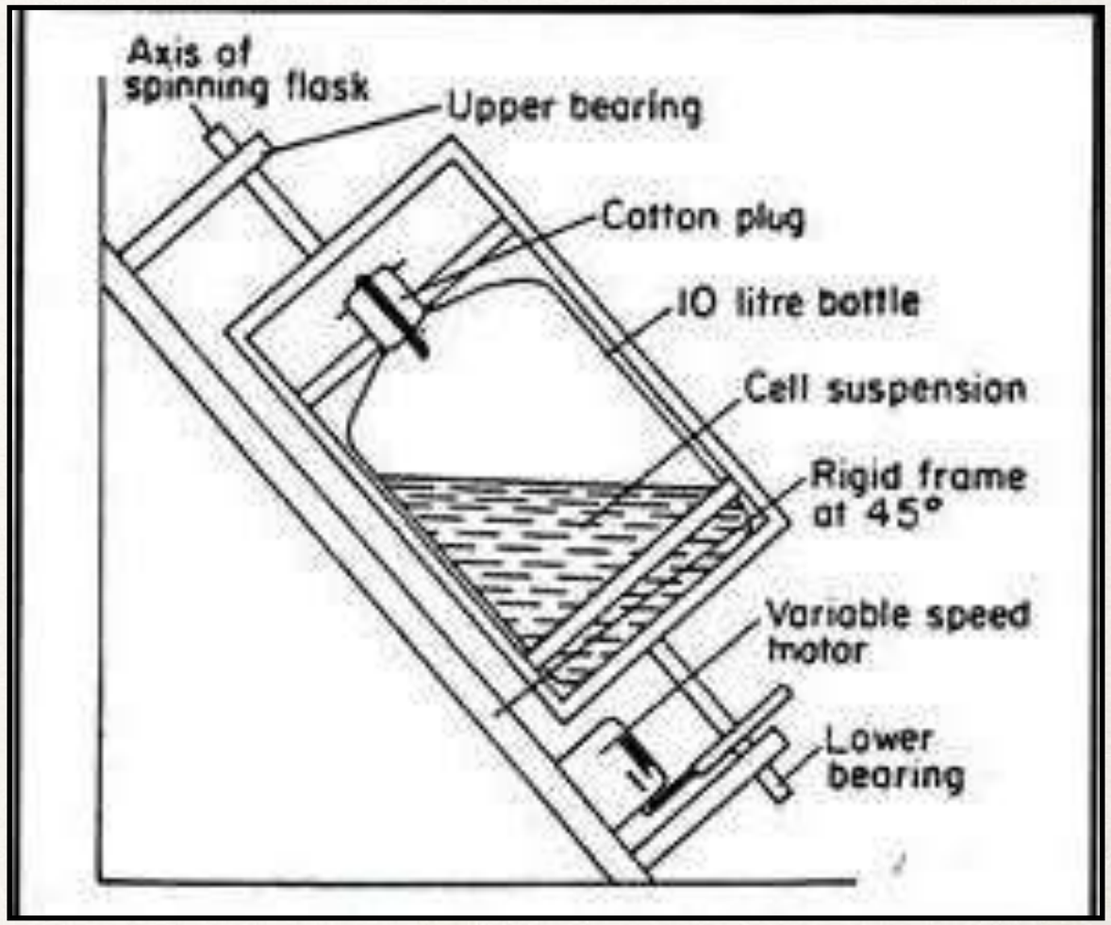
- It is very and effective system.

- In this method, single cells and cell aggregates in fixed volume of liquid medium are placed in conical flasks.
- These flasks are then mounted with the help of clips on a horizontal large square plate of an orbital platform shaker.
- The square plate moves in a circular speed of 60-180 rpm.



Spinning Culture

- In this culture system, large bottles are used, usually with a capacity of 10L.
- Large volumes of cell suspension is cultured in 10L bottles, with the bottles spinning in a spinner at 120 rpm at an angle of 45°.



Stirred culture

- This system is used for large scale batch culture.
- In this method, the large culture vessel (round-bottom flask) is not rotated but the cell suspension inside the vessel is kept dispersed continuously by bubbling sterile air through the culture medium.
- Internal magnetic stirrer is used to agitate the culture medium safely.
- The magnetic stirrer revolves at 200-600 rpm

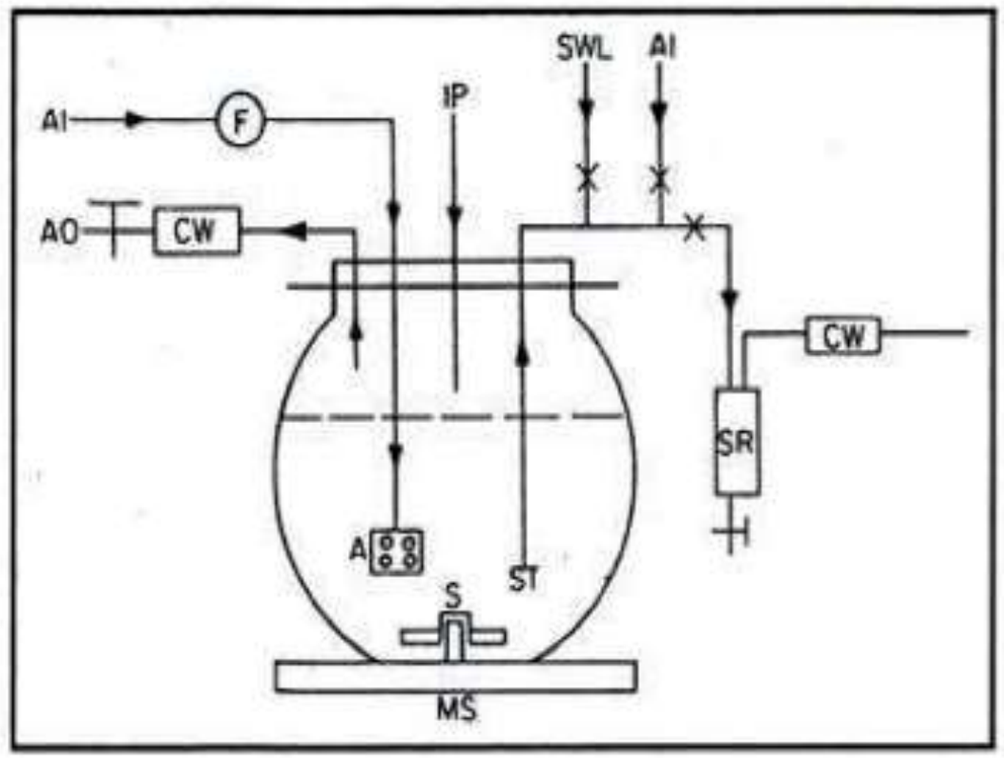


Fig 4.6

Stirred batch culture unit. Arrow indicate direction of flow of air; AI = air input; F = sterilizing glass-fibre air filter; AO = air outlet; CW = cotton wool; IP = inoculation port; A = aerator; S = stirrer magnet; ST = sample tube; MS = magnetic stirrer; SWL = sterile water line; SR = sample receiver; (Diagram after Dr. P. King)

Continuous culture

- In continuous culture system, the old liquid medium is replaced continuously by the fresh liquid medium to stabilize the physiological states of the growing cells.

- In this system, nutrient depletion does not occur due to the continuous flow of nutrients and the cells always remain in the steady growth phase.

- Continuous culture is further divided into two types :
  In closed type, the used medium is replaced with the fresh medium, hence, the cells from used medium are mechanically retrieved and added back to the culture and thus, the cell biomass keeps increasing.

- In open type, both the cells and used medium are replaced with fresh medium thus maintaining culture at constant and submaximal growth rate.

- Open continuous cell suspension culture is of two types :

- **<u>Chemostat :</u>**

  - In this system, culture vessels are usually cylindrical or circular in shape and possess inlet and outlet pores for aeration and the introduction and removal of cells and medium.

  - Such a system is maintained in steady state.

  - Thus in steady state condition the density, growth rate, chemical composition and metabolic activity of the cells all remain constant.

  - Such continuous cultures are ideal for studying growth kinetics and the regulation of metabolic activity in higher plants.

Turbidostats :

- A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed.

- In this system, the cells are allowed to grow upto a certain turbidity, when the predetermined volume of culture is replaced by fresh culture.

- The turbidity is measured by the changes of optical density of medium.

- An automatic monitoring unit is connected with the culture vessel and such unit adjusts the medium flow in such a way, as to maintain the optical density or pH at chosen, present level.

Media Components

- Macronutrients
- Micronutrients
- Carbon and energy sources
- Vitamins and myoinositol
- Amino acids
- Growth regulators

MS - Murashige and Skoog

G5 - Gamborg et al

W - White

LM - Lloyd and McCown

VW - Vacin and Went

Km - Kudson modified

M - Mitra

NN - Nitsch and Nitsch media

Medium Components ($mg.l^{-1}$)	MS	G5	W	LM	VW	Km	M	NN
Macronutrients								
$Ca_3(PO_4)_2$					200.0			
NH_4NO_3	1650.0			400.0				720.0
KNO_3	1900.0	2500.0	80.0		525.0	180.0	180.0	950.0
$CaCl_2.2H_2O$	440.0	150.0		96.0				166.0
$MgSO_4.7H_2O$	370.0	250.0	720.0	370.0	250.0	250.0	250.0	185.0
KH_2PO_4	170.0			170.0	250.0	150.0	150.0	68.0
$(NH4)_2SO4$		134.0			500.0	100.0	100.0	
$NaH_2PO_4.H_2O$		150.0	16.5					
$CaNO_3.4H_2O$			300.0	556.0		200.0	200.0	
Na_2SO_4			200.0					
KCl			65.0					
K_2SO_4				990.0				
Micronutrients								
KI	0.83	0.75	0.75			80.0	0.03	
H_3BO_3	6.20	3.0	1.5	6.2		6.2	0.6	10.0
$MnSO_4.4H_2O$	22.30		7.0		0.75	0.075		25.0
$MnSO_4.H_2O$		10.0		29.43				
$ZnSO_4.7H_2O$	8.6	2.0	2.6	8.6			0.05	10.0
$Na_2MoO_4.2H_2O$	0.25	0.25		0.25		0.25	0.05	0.25
$CuSO_4.5H_2O$	0.025	0.025		0.25		0.025		0.025
$CoCl_2.6H_2O$	0.025	0.025				0.025		
$Co(NO_3)_2.6H_2O$							0.05	
Na_2EDTA	37.3	37.3		37.3		74.6	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8		27.8		25.0	27.8	27.8
$MnCl_2$						3.9	0.4	
$Fe(C_4H_4O_6)_3.2H_2O$					28.0			
Vitamins and other supplements								
Inositol	100.0	100.0		100.0				100.0
Glycine	2.0	2.0	3.0	2.0				2.0
Thiamine HCl	0.1	10.0	0.1	1.0		0.3	0.3	0.5
Pyridoxine HCl	0.5		0.1	0.5		0.3	0.3	0.5
Nicotinic acid	0.5		0.5	0.5			1.25	5.0
Ca-panthothenate			1.0					
Cysteine HCl			1.0					
Riboflavin						0.3	0.05	
Biotin							0.05	0.05
Folic acid							0.3	0.5

Advantages

- Compounds can be produced under controlled conditions as per market demands.
- Culture systems are independent of environmental factors, seasonal variation, pest and microbial diseases and geographical constrains.
- Cell growth can be controlled to facilitate improved formation.
- The quality of the product will be consistent as it is produced by specific cell line.
- Recovery of product will be easy.
- Plant cultures are particularly useful in case of plants which are different or expensive to be grown in fields.
- The production time is less and labour cost are minimal.

Limitations

- The yield obtained in invivo production is lower when compared to intact plant.

- Cultured cells are genetically unstable and may undergo mutations. The production of secondary metabolites reduces drastically, as the culture ages.

- Strict aseptic conditions have to be maintained during culturing technique.

Cryopreservation

- Cryopreservation is a non-lethal storage of biological material at **ultra-low** temperature. At the temperature of liquid nitrogen (-196 degree) almost all metabolic activities of cells are ceased and the sample can then be preserved in such state for extended periods.

- However, only few biological materials can be frozen to (-196 degree) without affecting the cell viability.

INTRODUCTION

- In recent years with tremendous increase in population:

- Forest
- Land resources
- Population of medicinal plants
- Aromatic plants species

- **Liquid nitrogen** is most widely used material for cryopreservation.
- Dry ice can also be used.

- **Why Liquid nitrogen ?**

✓ Chemically inert

✓ Relatively low cost

✓ Non toxic

✓ Non flammable

✓ Readily available

STORAGE

THAWING

DETERMINATION OF SURVIVAL OR VIABILITY

STORAGE

- The frozen cells/tissues are kept for storage at temperature ranging from -70 to -196°c.
- Temperature should be sufficiently low for long term storage of cells to stop all the metabolic activities and prevent biochemical injury.
- Long term storage is best done at -196°c.

THAWING

- It is done by putting ampoule containing the sample in a warm water bath (35 to 40°c).
- Frozen tips of the sample in tubes or ampoules are plunged into the warm water with a vigorous swirling action just to the point of ice disappearance.
- It is important for the survival of the tissue that the tubes should not be left in the warm water bath after ice melts .

DETERMINATION OF SURVIVAL:

- Regrowth of the plants from stored tissues or cells is the only test of survival of plant materials.
- Various viability tests include Fluorescien diacetate (FDA) staining , growth measurement by cell number , dry and fresh weight.
- Important staining methods are:
- Evan's blue staining.

EVAN'S BLUE STAINING

- One drop of 0.1% solution of Evan's blue is added to cell suspension on a microscope slide and observed under light microscope.
- Only non viable cells (dead cells) stain with Evan's blue. % of viable cells = Number of fluorescent cells × 1oo total no of cells(viable + non-viable).

APPLICATIONS OF CRYOPRESERVATION:

- Freezing of cell cultures.
- Maintenance of disease free stock
- Seed Bank
- Gene Bank
- Storage of rare germplasm.

Techniques:

1. **Collection and preservation of cells & tissues**
2. **Addition of cryoprotectants**
   - Vitrification.
   - Cryoprotective dehydration.
3. **Freezing**
   - Slow freezing method.
   - Rapid freezing method.
   - Step wise freezing method.
4. **Storage**
5. **Thawing**

Collection Of Cells:

What can cryopreserved?

- In general, cryopreservation is easier for thin samples and small clumps of individual cells, because these can be cooled more quickly and so require lower doses of toxic cryoprotectants.
- Therefore, the goal of cryopreserving human livers and hearts for storage and transplant is still some distance away.
- Nevertheless, suitable combinations of cryoprotectants and regimes of cooling and rinsing during warming often allow the successful cryopreservation of biological materials, particularly cell suspensions or thin tissue samples like.

1. Semen .
2. Blood .
3. Stem cell.
4. Umbilical cord blood.
5. Egg (Oocytes).
6. Embryo (2,4 or 8 cell).
7. Plant (Seed & Shoot).

Addition of Cryoprotectant:

- There are two potential sources of cell damage during cryopreservation.
- 1. Formation of large ice crystals inside the cell.
- 2. Intracellular concentration of solutes increase to toxic levels before or during freezing as a result of dehydration.
- Cryoprotectants acts like antifreeze, they lower freezing
- temperature and increase viscosity.

Benefits Of Vitrification:

Vitrification:-

- It is the process in which ice formation can't take place because the aqueous solution is too concentrated to permit ice crystals nucleation, instead, water solidified into an amorphous 'Glassy' state.
- Rapid cooling also promotes vitrification.

Cryoprotective Dehydration:-

- If cells are sufficiently dehydrated they may be able to withstand immersion in liquid nitrogen. Dehydration can be achieved by growing in presence of high concentration of osmotically active compounds like sugars, polyols and / or air desiccation in a sterile flow cabinet or over silica gel.
- Dehydration reduces the ice formation,increases the osmotic pressure of the intracellular solution (the cytoplasm) which depresses its freezing temperature.
- Various cryoprotectants used are glycerol, dimethylsulphoxide, mannitol, propylene, choline etc.

Freezing:

A. Slow Freezing Method(SFM):-

- The tissues is slowly frozen with decrease in temperature of -0.5°C to -5°C/ min from 0°c-100°c, and then transfer to liquid nitrogen.

B. Rapid Freezing Method(RFM):-

- The material is plunged into liquid nitrogen decreases in temperature from -300°C to -1000°C/min or more, the quicker the freezing is done the smaller the ice is crystal.

C. Stepwise-Freezing Method:-

- In this method slow freezing down to -20°C to -400°C ,a stop for a period of approximately 30 min and then additional rapid freezing to -196°C is done by plunging in liquid nitrogen.

Storage:

- Storage of frozen material at the correct temperature is as important as freezing.

  In general the frozen cells/tissues are kept for storage at a ranging from -70°C to -196°C.

- However, with temperature above -130°C ice crystal grow may occur, inside the cells which reduces viability of cell. Storage is ideally done in liquid nitrogen refrigerator at -150°C in vapor 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.
- For long term storage temperature at -196°C in liquid nitrogen is ideal.
- A regular & constant supply of liquid nitrogen to refrigerator is essential.

Thawing:

- It is done by putting the ampoule containing the sample in a warm water (35°to 45°C)bath.
- By this approach, rapid thawing [at the rate of (500 to&750°C/min) occurs, and the protects and this protects the cell from damaging effect of ice crystal formation.
- As the thawing occurs (ice completely melts the ampoule are quickly transferred to water bath at temperature 20 to 25°C .
- This transfer is necessary since the cells get damaged if left for long in warm (37°C - 45°C) water.
- For cryopreserved material(cells/tissues) where the water content has been reduced to an optimal level before freezing, the process of thawing become less critical.

Benefits and Disadvantages:

Benefits:

- It is useful in the breeding of dairy cattle, pigs & dogs.
- The cryopreserved blood can stored for many years & transfuse to required person.
- The cancer & tumor cells can be preserved for further research.
- Stem cells, Umbilical cord blood , skin cells are preserved for further use.
- It is helpful for endangered animals & plants (medicine & fragrant).
- It is quite safe for “IVF” because the donor is retested for HIV .

Disadvantages:

- High cost.
- Social issues.

Cell Banking

- A **cell bank** is a facility that stores **cells** of specific genome for different purposes.
- The first person accredited with making a cell bank for widespread use was **Kral,** a Czechoslovakian scientist who created his cell bank collection in the late 1890s.

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.

♦ Germplasm conservation refers to maintain the collected germplasm in such a state that there is minimum risk for its loss and that either it can be planted directly in the field or it can be prepare for planting with relative ease when ever necessary.

♦ 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.


RICE GERMPLASM
CONSERVATION PLOT
NO. OF GERMPLASMS-108
DATE OF SOWING - 27/06/11
DATE OF TRANSPLANTING - 25/7/11
SPACING 10 X 20 CM

- A global body namely International Board of Plant Genetic Resources (IBPGR) has been established for germplasm conservation. Its main objective is to provide necessary support for collection, conservation and utilization of plant genetic resources throughout the world.

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

1. In-situ conservation
2. Ex-situ conservation

1) In – situ Conservation:

- Conservation of germplasm under natural habitat is referred to as in situ conservation. It requires establishment of natural or biosphere reserved national parks or protection of endangered areas or species. In this method of conservation, the wild species and the complete natural or semi natural ecosystem are preserved together.

- A gene sanctuary is best located with the centre of origin of crop species concerned, preferably covering the microcenter with in the centre of origin. NBPGR, New Delhi is making attempts to establish gene sanctuaries in Meghalaya for Citrus and in the North-Eastern region for Musa, Citrus, Oryza, Saccharum and Megifera.

In situ conservation

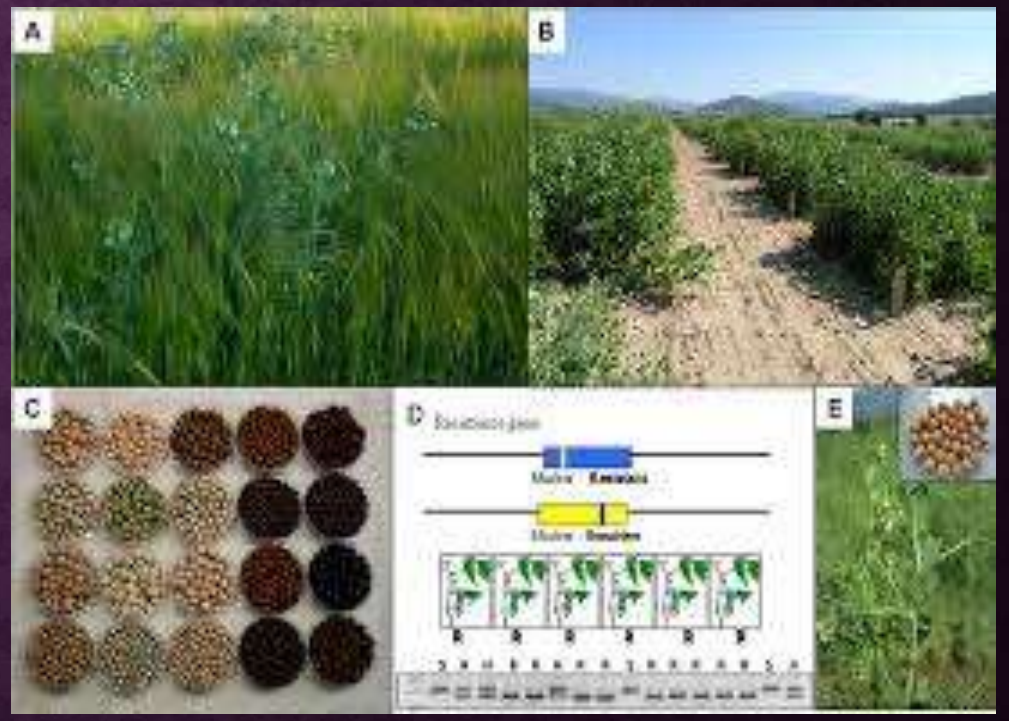
Biosphere reserves are another category of protected areas.

A large area is declared as a biosphere reserve where wildlife is protected, but local communities are permitted to continue to live and pursue traditional activities within the reserve.

Indian government has setup 7 biosphere reserves:

Biosphere Reserves- Nokrek

Biosphere Reserves- Nilgiri



Images of in situ conservation

In situ banana conservation

♦ Gene sanctuaries offer the following two advantages:

1. A gene sanctuary not only conserves the existing genetic diversity present in the population, it also allows evolution to continue. As a result, new alleles and new gene combinations would appear with time.

2. The risks as sociated with ex situ conservation are not operative.

♦ This method of preservation has following main disadvantages.

1. Each protected are will cover only very small portion of total diversity of a crop species, hence several areas will have to be conserved for a single species.

2. The management of such areas also poses several problems.

3. This is a costly method of germplasm conservation.

2) Ex-Situ Conservation:

- Conservation of germplasm away from its natural habitat is called ex situ germplasm.
- This is the most practical method of germplasm conservation.
- This method has following three advantages:

1.It is possible to preserve entire genetic diversity of a crop species at one place.
2. Handling of germplasm is also easy.
3. This is a cheap method of germplasm conservation.

Ex situ conservation

Images of ex situ conservation

- The germplasm is conserved either 1) In the form of seed. Or 2) In the form of meristem cultures.
- Preservation in the form of seed is most common and easy method. Seed conservation is relatively safe, requires minimum space (except coconut, etc) and easy to maintain .
- Glass, tin or plastic containers are used for preservation and storage of seeds.
- The seeds can be conserved under long term (50 to 100 years), medium term (10-15 years) and short term (3-5 years) storage condition.
- Roberts (1973) has classified seeds into two groups for storage purpose, viz.

1) orthodox and
2) Recalcitrant.

1. Orthodox:

♦ Seeds which can be dried to low moisture content and stored at low temperature without losing their viability are known as orthodox seeds.

♦ This group includes seeds of corn, wheat, rice, carrot, beets, papaya, pepper, chickpea, lentil, soybean, cotton, sunflower, various beans, egg plant and all the Brassicas. These seeds can be dried and stored at low temperatures for long periods of time.

2. Recalcitrant:

♦ Seeds which show very drastic loss in viability with a degree in moisture content below 12 to 13% are known as recalcitrant seeds . This group includes cocoa, coconut, mango, tea, coffee, and rubber, jackfruit, and oil palm seeds. Such seeds cannot be conserved in seed banks and therefore, require in situ conservation.

♦ Crop species with recalcitrant seeds are conserved in field gene banks which are simply areas of land in which collections of growing plants are assembled . For conservation of meristem cultures, meristem cultures, meristem or shoot tip banks are established .

Conservation of genetic stocks by meristem cultures has several advantages as given below:

1. Exact genotype can be conserved indefinitely free from virus or other pathogens and without loss of genetic integrity.

2. It is advantages for vegetatively propagated crops like potato, sweet potato, cassava, etc, because seed production in these crops is poor.

3. Vegetatively propagated material can be saved from natural disasters or pathogen attack.

4. Long regeneration cycle can be envisaged from meristem cultures.

5. Perennial plants which take 10-20 years to produce seeds can be preserved any time by meristem cultures.

6. Regeneration of meristem is extremely easy.

7. Plant species having recalcitrant seeds can be easily conserved by meristem cultures.

Germplasm Activity – Evaluation

Evaluation refers to screening of germplasm in respect of morphological genetical, economic, biochemical, and physiological, pathological, and entomological attributes. Evaluation of germplasm is essential from following angles.

1. To identify gene source for resistance to biotic and abiotic stresses, earliness, dwarfness, productivity, and quality characters.

2. To classify the germplasm into various groups.

3. To get a clear picture about the significance of individual germplasm line.

Evaluation requires a term of specialists from the disciplines of plant breeding, physiology, biochemistry, pathology and entomology. First of all a list of descriptors (Characters) for which evaluation has to be done is prepared. This task is completed by a team of experts from IPGRI, Rome, Italy. The descriptions are ready for various crops.

Large number of accessions is available in maize, rice, wheat, sorghum, potato, and other major crop. About 7.3 million germplasm accessions are available in 200 crops species.

Handling of such huge germplasm information is only possible through electronic computers. For uniformity of information IPGRI has designed descriptors (characters) and descriptor state for majority of crops.

The entire data is put in the computer memory and the desired information can be obtained any time from the computer.

The material is evaluated at several locations to get meaningful results. Moreover, evaluation is done in a phased manner. The variation for polygenic character is assessed by three different methods as given below:

1. By simple measures of dispersion (range, standard deviation, standard error and coefficient of variation)

2. By metroglyph analysis of Anderson (1957) and

3. By D2 statistics of P.C Mahalanobis (1936).

The evaluation of germplasm is down in three different places, viz. 1) In the field, 2) In green house, and 3) In the laboratory, observation on morphological characters, parameters like photosynthetic efficiency and transpiration rate can be recorded under field conditions using portable instruments.

The resistance to biotic and abiotic stresses can also be screened under green house conditions. Evaluation for biochemical characters like protein, oil and amino acid contents, and technological character is competed under laboratory conditions. Both visual observation and metric measurements are used for evaluation.

Germplasm Activity – Documentation

Documentation refers to compilation, analysis, classification, storage and dissemination of information.

In plant genetic resources, documentation means dissemination of information about various activities such as collections, evaluation, conservation, storage and retrieval of data.

Now the term documentation is more appropriately known as information system.

Documentation is one of the important activities of genetic resources. Information system is useful in many ways as given below:

1. It provides information about various activities of plant genetic resources.

2. It provides latest information about characterization conservation, distribution, and utilization of genetic resources.

3. It helps explores, evaluators and curators in the conservation of genetic resources.

4. It helps in making genetic resources accessible to plant breeders and other users.

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Germplasm Activity – Distribution

The distribution of germplasm is one of the important activities of genetic resources centres. The specific germplasm lines are supplied to the users on demand for utilisation in the crop improvement programmes.

1. Distribution of germplasm is the responsibility of the gene bank centres where the germplasm is maintained and conserved.

2. The germplasm is usually supplied to the workers who are engaged in the research work of a particular crop species.

3. Germplasm samples are generally supplied free of cost to avoid cumbersome work of book keeping.

4. The quantity of seed samples to be sent is usually small; depend on the availability of seed material and demands received for the same and several other factors.

5. Proper records are maintained about the important characters of the accessions to the distributor who will record the information in the germplasm register for documentation purpose.

6. The germplasm is usually distributed after evaluation by collecting centres for one or two crop seasons. It helps in acclimatization and purification of the material.

7. Without distribution to the actual users, there is no point in collecting the germplasm.

Germplasm Activity – Utilization :

are briefly discussUtilization refers to use of germplasm in crop improvement programmes. The germplasm can be utilized in various ways. The uses of cultivated and wild species of germplasm ed below:

Cultivated Germplasm:

The cultivated germplasm can be used in three main ways:
1) As a variety, 2) as a parent in the hybridization, and 3) as a variant in the gene pool. Some germplasm lines can be released directly as varieties after testing.

If the performance of an exotic line is better than a local variety, it can be released for commercial cultivation. In some cases, new variety is developed through selection from the collection. Some germplasm lines are not useful as such, but have some special characters, such as disease resistance, good quality of economic produce, or wider adaptability.

These characters can be transferred to commercial cultivars by incorporating such germplasm lines in the hybridization programmes. Transfer of desirable character from cultivated germplasm to the commercial cultivars is very easy because of cross compatibility.