SEMESTER - VI

PAPER - VI

TISSUE CULTURE AND BIOTECHNOLOGY

UNIT-I

PLANT TISSUE CULTURE

The technique of growing of plants by culturing the cells or tissues or an organ on artificial nutrient medium under aseptic environment is called as **"plant tissue culture"**. Plant tissue culture was introduced by G. Haberlandt for the first time in the year 1905. Each plant cell has genetic information to form new cells. This inherent capacity of the cell is known as **"totipotency"**. The process of production of new plants on artificial nutrient medium is known as in vitro culture. Plant tissue culture technique consists of six steps. They are

1. Preparation of culture medium
2. Sterilization of culture medium
3. Preparation of explants
4. Inoculation of explants on to medium
5. Incubation for the growth.
6. Transfer of acclimatized plants to the pots.

1. Preparation of culture medium:

Culture medium is the mixture of various essential nutrients in required proportion. Medium consists of inorganic compounds organic compounds and growth regulators. Agar is used as solidifying agent.

a. Inorganic Compounds: Various types of inorganic nutrients are essential for the growth of tissue. Macro elements such as N, P, K, Ca, Mg, S and micro elements like Fe, Mn, Zn, Mo, B are essential for the growth of plant cells.

b. Organic Compounds: Organic compounds which added to the culture medium are amino acids, vitamins and carbohydrates. Glycine is the most commonly used amino acid. Thiamin (Vit - B_1) is the vitamin more frequently used in tissue culture. Sucrose is more commonly used carbohydrate. The culture medium is also fortified with coconut milk, yeast extract and fruit juices (tomato and water melon). Coconut milk provides cytokinins. Yeast extract provide nitrogen and vitamins.

c. Growth regulators: The culture medium supplemented with growth regulators like auxins, gibberellins and cytokinins. Auxins induce cell division and root formation. Auxins like IAA, IBA, NAA and 2, 4-D are most commonly used in tissue culture. Gibberellins are rarely used. But GA3 is used in shoot tip culture. Cytokinins induce cell division and shoot differentiation.

A medium with inorganic compounds, vitamins, sucrose and without growth regulators is called as minimal or basal medium. The minimal medium which is added by amino acids and growth regulators is known as artificial medium. All the constituents are dissolved in distilled water, the final pH of the medium solution is adjusted to 5.6 to 6.0. Agar - Agar is used for the solidification of the medium.

According to the purpose, the medium may be taken in test tubes or flasks. These containers have to be closed with non-absorbent cotton plug to facilitate the exchange of oxygen and carbon dioxide.

2. Sterilization of the medium:

The medium is rich in nutrients. Hence a variety of micro organisms especially bacteria and fungi grow and spoil the medium. Therefore the medium needs to sterilize. Sterilization is carried out by different methods. However, autoclave sterilization, is most commonly used. Autoclaving at 15 lb (pounds) pressure (121°C) for 15 minutes completely sterilizes the medium.

Culture vessels are first sterilized with 95% ethyl alcohol and then kept in oven at 160 - 180 for 3- 4 hours. The plant material (explants) is washed thoroughly with a liquid (eg. 5% Teepol) for 15 minutes. Then it is thoroughly washed with sterile distilled water. After that, the material is

sterilized with sodium hypochlorite and sterile distilled water, The inner surface of the inoculation chamber is sterilized through UV radiation.

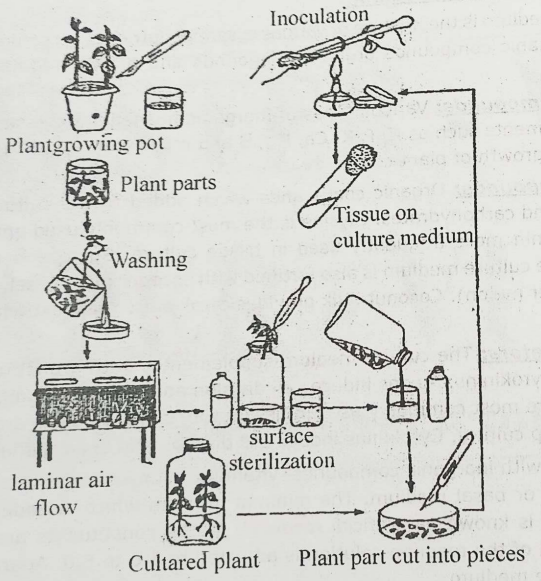
3. Explant Preparation:

Any plant part which is used as inoculum to grow the plant in vitro is called **"explant"**. Any part of the plant such as root, stem, shoot tip, meristematic tissue, parenchyma, anthers etc... can be used as explants.

4. Inoculation of explant on to medium:

The transfer of surface sterilized explants on to the medium is known as inoculation. Inoculation is carried out within a **'laminar air flow chamber'** which maintains the aseptic conditions.

5. Incubation for growth:



After inoculation, the culture vessels incubated in culture room where there is provision for maintenance of ideal temperature (25°C), light and humidity.

When cultures are incubated for 3-4 weeks, the cells of the explant absorb the nutrients, grow and undergo repeated divisions to produce a proliferating undifferentiated mass of cells known as **'callus'**. On minimal/basal medium only callus will be produced. Callus form organs like root, shoot and thus complete plants. This process is known as **'organogenesis'**. Generally auxins induce root formation and cytokinins shoot formation.

6. Transfer of acclimatized plants to the pots:

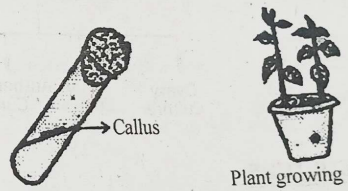
The plants thus developed from the explants are acclimatized and transferred to pots. The plants are washed with water to remove medium. These plants are then transferred to pots. These

pots are covered with polythene bags and maintained at room temperature for one to two weeks. Healthy and strong plants are then transferred to large pots containing the mixer of common soil and compost.

MICRO - PROPAGATION

Multiplication of genetically identical copies of a plant through in vitro clonal propagation is called **'micro-propagation'**. Generally clonal propagation is used to produce plants which do not produce seeds through sexual reproduction. Plants like apple, potato and ornamental plants are developed through this method. This method was first proposed by G. Morel in the year1960."He used this method for the production ornamental plants such as orchids. The main objective of this method is to produce more plants in limited time and space.

PROCEDURE:-



Micro propagation involves 5 stages. First stage involves the selection and growth of stock plants for about 3 months under controlled conditions. In second stage, selection and culture of appropriate explant on a suitable medium is achieved. The most commonly used explants are shoot tips and axillary buds. The chosen explants are surface sterilized before use. Third stage involves the formation of callus from the cultured explants. In fourth stage, medium is to be supplemented with growth regulators. In general auxins induce roots and cytokinins induce shoots from the callus. Last stage involves the establishment of regenerated plantlets in the sterilized soil within the pots. This is followed by transferring the plantlets from laboratory to green house. Plantlets are maintained in the green house for few days. During this period plantlets become harder and acclimatized. After that the plants are cultivated in the natural environment.

Advantages:

The technique of micropropagation is an alternative approach to conventional methods of vegetative propagation. Micropropagation has more benefits.

Benefits:

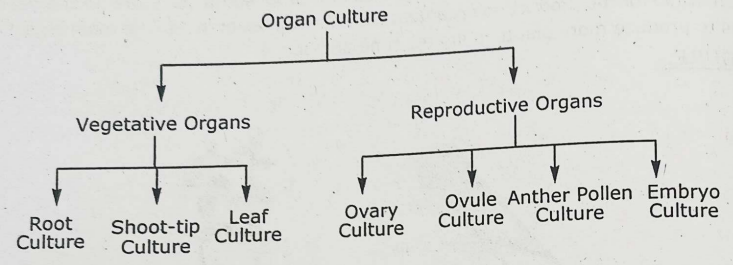
1. A large number of plants can be produced within a short time and space throughout year and exported to other countries.
2. Disease free plants can be developed through micropropagation. Disease free plants can be produced through shoot tip culture.
3. Artificial seeds can be produced through the encapsulation of axillary buds. These synthetic seeds show more genetic variations. Eg: Cauliflower, Onion.
4. More number of plants can be produced through micropropagation within limited time, space and with low investment. Millions of plants can be produced in a small room by utilizing limited human resources.
5. Now a day's automatization have also been used for the development of plants especially shoots and bulbs in micro propagation. This reduces the cost of labour component in

micropropagation.

ORGAN CULTURE

Culturing of plant organs on nutrient medium is called organ culture. Organ culture provides an excellent opportunity to define the nutrients and growth factors normally received by the organs.

On the basis of the origin of the explant, organ culture can be categorised into different types, which are given below.



VEGETATIVE ORGAN CULTURE

1. ROOT CULTURE:

Root culture can be defined as the culture. of excised radical tips of seedlings in a liquid medium.Root tips of the plants are not suitable for culture because the roots of the plant are buried deeply in the soil. Root tips are very sensitive to disinfectants. So it is better to avoid the surface sterilization of young root tips for the establishment of root cultures. Root cultures can be successfully initiated from the excised radical tips of aseptically germinated seeds.

- Seeds are surface sterilized and germinated on moist filter paper at 25° C in the dark.
- When the seedling roots are 20-40 mm in length apical tips are excised with help of scalpel.
- Excised root tips are transferred to liquid medium contained in test tubes.
- Test tubes are incubated at 25° C in the dark.

Applications:

- Root cultures have increased our knowledge of carbohydrate metabolism and role of mineral ions, vitamins etc. in root growth.
- Root cultures have provided basic information regarding the dependence of roots on shoots for growth regulators.

2. LEAF CULTURE:

Culture of excised young leaf primordia or young leaf of the shoot apex on nutrient medium is known as leaf culture.

Procedure: Detach the vegetative bud or very young leaf from shoot apex. Wash the explants thoroughly with running tap water. Then vegetative bud or young leaves are surface sterilized with 70% ethyl alcohol for 30 seconds. This is followed by 10-15 minutes incubation in 0.8% sodium hypochlorite. Excise the leaf primordial from the bud with the help of scalpel. Later inoculate the leaf primordia onto solidified medium in test tubes and incubate the cultures at 25° C under 16 hrs photo period.

Advantages/ Importance: Leaf cultures are valuable to study the effects of growth regulators and environmental conditions on leaf development.

3. SHOOT TIP (MERISTEM CULTURE) CULTURE:

The culture of terminal portion of a shoot comprising the meristem together with leaf primordia is known as shoot tip culture. In vitro culture of shiny dome like structure with one or two pairs of the leaf primordia is known as. meristem culture.

Procedure:

- Select and remove the young twigs from a healthy plant. Cut the 1 cm tip portion of the twig."Surface sterilizes the shoot tips by incubation in a sodium hypochlorite solution. Then shoot tips washed with sterile distilled water.
- Transfer the shoot tips to a sterilized petri dish and remove the outer leaves from shoot tips. Transfer the shoot tips onto agar solidified medium and incubate the cultures under 16hrs light at 25° C.
- As soon as the shoots obtained from the shoot tip or meristem, develop roots, transfer them to hormone free medium.
- The plantlets formed from shoot apices are later transferred to pots containing compost.

Applications:

1. Virus free plants can be produced through shoot tip culture.
2. Shoot tip culture of many plants can be used for micro propagation.

REPRODUCTIVE ORGAN CULTURE:

1. ANTHER CULTURE (POLLEN CULTURE):-

Anther culture is the culture of developing anthers isolated from unopened flower buds on a nutrient medium. Pollen grains in the anther are haploid. During culture, pollen grains divide mitotically and produce haploid callus and haploid plantlets. The technique of culture of uninucleate pollen grains on nutrient medium is known as **"pollen culture"**.

Procedure:

- The young flower buds 17-22mm in length are collected and surface sterilized by immersing in 70% ethanol for 10 seconds then in sodium hypochlorite for 10 minutes. After that flower buds are washed with sterile distilled water and transfer into a sterile petridish.
- With the help of sharp scalpel and using forceps the buds are split open and anther lobes are taken out. Collected anther lobes are placed into agar solidifies medium. The cultures are incubated at 24° -28°C in dark for 3-4 weeks. The haploid plantlets develop, comes out by bursting the anther lobes. At this stage the cultures are incubated at 24° -28°C under 14 hrs light.
- Plantlets are removed and sub-cultured on suitable media for further and root development.
- When the plantlets are about 50mm tall, removed from the medium and washed with tap water and then transferred to small pots having compost.

Applications:

1. Haploid plants can be produced through anther culture and pollen culture. These haploids are used in genetic researches.

2. Haploid are used to produce homozygous diploids by duplicating the number of chromosomes.
3. By producing the haploids, reduce the breeding time and can obtain various genetic combinations.

2. OVULE CULTURE:

In vitro culture of isolated ovules from the ovary on chemically defined nutrient medium is called as **"ovule culture"**.

Procedure:

- Collect the open flowers. Remove sepals, petals, androecium etc. and collect ovaries.
- Soak the ovaries in 6% sodium chloride solution. Then rinse the ovaries with sterile distilled water.
- Ovules are gently prodded with the help of spoon shaped spatula by breaking the funicles at its junction with placental tissue. Later ovules are transferred into agar solidified medium or liquid medium. Incubate the ovule cultures at 25° C for under16 hrs light.

Applications:

1. Virus free stock plants from the diseased plants can be produced.
2. It helps in the development of polyembryony in Citrus and other plants,
3. Orchid seeds are germinated only in association with proper fugal hyphae. Cultured ovules (fertilized) of orchids successfully germinate without fungal hyphae.

3. EMBRYOCULTURE:

The culture of zygotic embryos of different developmental stages on nutrient medium is known as **"embryo culture"**.

Procedure:

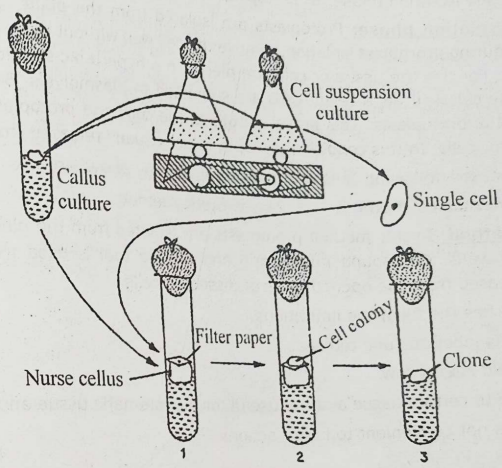
- Fruits are surface sterilized for 5 -10 minutes in 0.1% mercuric chloride. Wash repeated1y with sterile distilled water.
- The fruits are kept in a depression slide containing few drops of liquid medium.
- Remove the pericarp and cut the fruit into two equal halves. The halves are pushed apart with forceps to expose ovules.
- A small incision the ovule followed by slight pressure with blunt needle to free the embryos.
- The excised embryos are transferred petridishes containing medium. Usually 5 -10 embryos are cultured in a petridish.
- The petridishes are sealed with cello - tape. The cultures are incubating at 25°C for under16 hrs light. Gradually the cultured embryos are developing into plantlets. The plant-lets are transferred to pots containing compost and kept in green house for acclimatization.

Applications:

1. Seed dormancy can be removed through embryo culture.
2. Metabolic and biochemical aspects of seed dormancy can be understood.
3. Abortive embryos can be rescued and made to develop into plants.

CALLUS CULTURE

1. CELL CULTURE:



Paper Raft Nurse technique

Culture of isolated cells on nutrient medium under aseptic conditions is called as cell culture.

- Cells are isolated from the tissue.
- Few day before cell isolation filter paper rafts are prepared. For this filter paper is cut into small square shaped pieces. These filter paper pieces are placed aseptically on the surface of growing callus. The callus which supports the filter paper is known as **"nurse tissue"**.
- The isolated cells are placed aseptically on filter paper raft. These isolated cells absorb nutrients and growth factor essential for the cell division from the nurse tissue.
- The whole cultures system is incubated at 25°C under 16 hrs photo period. During this period cell divisions are initiated and as a result cell colonies are formed.
- The above cell colonies are transferred onto culture medium. Gradually from these cell colonies calluses are formed. A callus tissue originates from a single cell is called as **"single cell clone"**.

APPLICATIONS:

1. Single cell cultures are used for the development of single cell generated clones.
2. It is possible to regenerate the whole plants from the callus formed by single cell cultures.
3. Single cell cultures are useful to study the mutations.

2. PROTOPLAST CULTURE:-

The in vitro culture of isolated protoplasts in nutrient medium is called as protoplast culture.

Procedure: The protoplast culture explained in phases.

A) Protoplast isolation phase, B) protoplast culture phase

A) Protoplast isolation phase: Protoplasts are isolated from the plant tissues. Proper care should be taken during protoplast isolation, remove the cell wall without damaging the concerned cell or protoplast. For this the tissue or cells are placed in a hypertonic solution (13% mannitol) before remove the cell wall. As a result cells are subjected to plasmolysis. Because of this, cell walls apart from the protoplasts. This process protects the cells and protoplasts. This results in osmotic stability for cells. In this condition protoplasts are usually released from the cells.

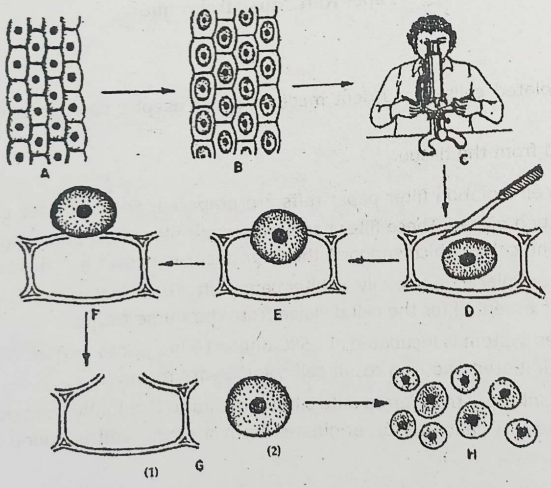
Two methods are following to isolate the protoplasts by above process. They are

1) mechanical method 2) enzymatic method

1) Mechanical method: In this method protoplasts are isolated from the plasmolyzed cells by dissecting the cell walls. Compound microscope and cutting tool is used for this technique. Protoplasts are released from the opened parts of dissected cells.

This method has the following limitations.

1) This method is laborious and tedious.
2) Protoplast yield is very low.
3) It is restricted to certain tissue and not useful for meristematic tissue and immature cells.
4) This method is not convenient to larger actions.



Isolation of protoplast by mechanical method

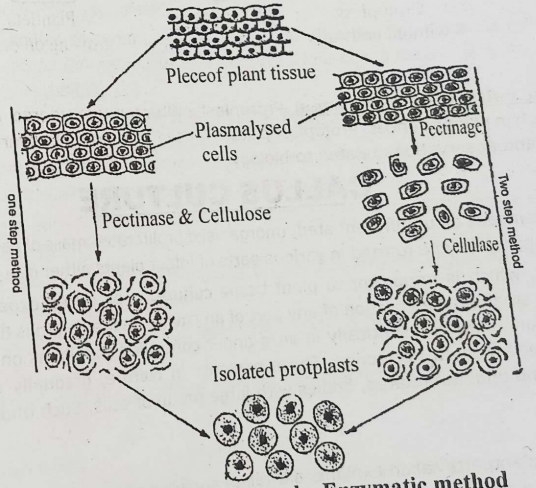
a) Piece of plant tissue b) Plasmolysed cells
c,d)Cutting of cell wall using compound microscope, scalpel
e,f) State of protoplast release g) (1) Empty cell (2) Proto plast
h) Isolated protoplasts

B) Enzymatic method: In this method cell walls are digested by using enzymes like cellulose, hemi cellulose, pectinase, macerozyme. This results in the releasing of protoplasts.

Protoplasts can be isolated from any part of the plant body by this method. However, mesophyll cells, pollen mother cells, pollen tetrads are most suitable for this method.

Process of protoplasts isolation:

1. Leaves are surface sterilized. For this 70% ethyl alcohol is used.
2. Leaves are then washed with sterile distilled water.
3. Small and thin epidermal pieces are separated from the leaf hypodermis.
4. Leaf pieces are subjected to plasmolysis by placing them in chemical mixture containing 13% mannitol. Later leaf pieces are subjected to shaking, using water bath shaker.
5. Above leaf pieces are transferred into enzyme mixture (pectinase/cellualse) after that, leaf pieces are incubated at 25° C for 24 hours.
6. The mixture in the above reaction is filtered and subjected to centrifugation (100g/m).
7. Protoplasts are separated from the bottom of centrifuge tubes and purify with sorbitol solution. Later protoplasts are placed in sucrose (20%) solution.
8. Above solution is once again subjected centrifugation (200 g/m). After that, protoplasts are separated with the help of pipette. Finally these protoplasts are transferred into cultured medium.



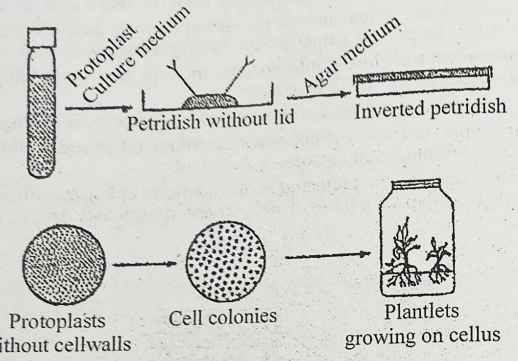
Isolation of protoplasts by Enzymatic method

B) Protoplast culture phase:

Isolated protoplasts are cultured in various methods. In which usually most frequently used method is agar-embedding technique.

An equal amount of protoplast suspension is added to the liquid culture medium having agar. Protoplast -agar mixture is poured in small petriplates. The technique of addition of protoplast suspension to agar solidified medium is called plating. The above mixture is made to be distributed evenly within the petriplates. Before this lids are fitted. on petriplates. Later protoplast-agar mixture is solidified and petriplates are inverted. After that the cultures are incubated at 25° C

under light. The cultures are repeatedly sub cultured in regular intervals. The developments and changes occur in protoplasts in cultured medium have been observed with the help of compound microscope. Protoplasts gradually develop cell wall and transforms into complete cells. These cells are called regenerated cells. Later, these cells divide, redivide and form cell colonies. These colonies are sub cultured on agar medium to produce new cultures. Callus formed in the final stage is induced to produce roots and shoots.



Protoplast culture - Advantages: Protoplast culture is considered as one of the major breakthrough in plant sciences. Protoplast culture is used as important experimental tool in various researches and observations related to biology.

CALLUS CULTURE

Callus means an undifferentiated, unorganised proliferated mass of cells. Sometimes callus or callus like parts may be formed in various parts of intact plants either due to wound or disease.

Callus, which is important to plant tissue culture, is produced experimentally from the explants. Any small excised portion of any part of any living healthy paltn is described as Explant. The explants are cultured aseptically in vitro under controlled conditions on a nutrient medium containing specific phyto hormones. These explants in culture, gradually develop in to large unorganised and undifferentiated, bodies with large group of cells. Such undifferentiated part is called Callus.

Procedure :

Excised plantparts called explants are taken and washed with liquid detergents (5% v/v - Teepol). Then the explants are surface sterilized by chemicals like Hg Cl_2 or sodium hypochlorite for 10–15 mts. and rinsed with auto claved distilled water. Such sterilized explant material is cut aseptically into small segments. These segments are transferred asepetically on a suitable nutrient medium (which is subjected to sterilization by auto clave at 15 lbs pressure for 15 mts) is used for the induction of callus tissue.

The above incubated Agar culture medium is transferred finally to the incubation culture rooms. Incubation should be done under controlled physical conditions. Generally 55% to 60% relative humidity, and $25^0C \pm 2^0C$ Temperatures are considered ideal for callus growth. Dark or light Condition is provided depending on the nature of the plant material. Photodependent plant

material normally require 16 Hrs. photo period.

After incubation, after the callus formation, portions of callus tissue are removed and transferred directly on to fresh nutrient medium to continue growth. These callus cultures can be maintained by serial subcultures.

Generally, different plant tissues of many plant, species can be used to induce callus formation. But Carrot roots are highly specific for callus Cultures. Generally callus Culture technique is described mainly, "Thro" the carrot root culture, which is taken as a typical example.

Importance of callus culture :

Callus Culture plays little role compared to other culture methods. But it is used in many other experimental fields, which help many areas of biology, to achieve the respective objectives or goals.

Callus culture shows its importance in certain areas like the following

1. Certain manipulations in the nutrients and hormones of callus culture medium help two phenomena like plant regeneration [PR] and somatic Embryogenesis (SE). It is possible to regenerate whole plant in large number from callus tissue (PR) Embryos can be produced directly from the somatic cells of callus tissue (SE).
2. Cell suspension culture can be initiated from callus culture.
3. Callus tissue is considered as a good source of Genetic variability. Plants with genetic variations can be regenerated from such modified callus cells.
4. Secondary metabolites of commercial value can be obtained from ccallus cultures. Direct extraction of a secondary metabolite or a drug from the callus tissue (ie used as source for such materials), is possible.

ORGANOGENESIS

- Organogenesis means the development of root, shoot, leaves, flowers either directly from an explant or from the callus culture is known as organogenesis.
- In this organogenesis process formation of plant organs occur through either meristematic or non meristematic tissues.
- In this process several tissues are organised together to form an organ, such as leaves, roots shoots, flowers & the vascular system.
- In plant tissue culture inducing organogenosis is an important way to regenerate plants from the culture.
- Organogenesis in plant tissue culture involves two distinct phases.

They are

i. Dedifferentiation ii. Redifferentiation

(i) **Dedifferentiation :** Dedifferentiation is a process by which cells develop in reverse, from a more differentiated to less differentiated state.

This process begins shortly after the isolation of the explant.

In this process, formation of callus from the explant tissue with accelerated cell division occurs.

(ii) **Redifferentiation :** Redifferentiation cause the development of primordia from a group of callus cells.

Redifferentiation may begin any time after the first callus cell forms.

- In this organogenesis process, first we have to sterilize the explant surface.
- After the sterilization process explant gets free from bacteria.

- Later on we have to take a test tube which contain nutrient medium.
- This medium consist plant growth regulators (PGR) like Auxins (24-D) and Cytokinins (Kinetin).
- Now we have to keep explant (cell) in to nutrient medium & close the test tube with using cotton.
- It will be incubated 2-3 weeks at 20-25^{0}C.
- During this incubation period explant under goes repeated cell divisions & forms undifferentiated mass of cells, which is known as callus.
- After formation of callus, callus takes major role to form root or shoot system.
- Here shoot development is known as cavlo genesis and root development known as Rhizogenesis.
- Either of both (Shoot (or) Root formation) only one process occurs.
- If Auxin concentration is low comparing to cytokinin to the nutrient medium, callus develops shoot system (Caulogenesis)

(or)

- If cytokinin concentration is low comparing to Auxin to the Nutrient medium, callus develops root system (Rhizogenesis)
- Here organogenesis will be of two types, they are (1) direct organogenesis (2) indirect organogenesis.

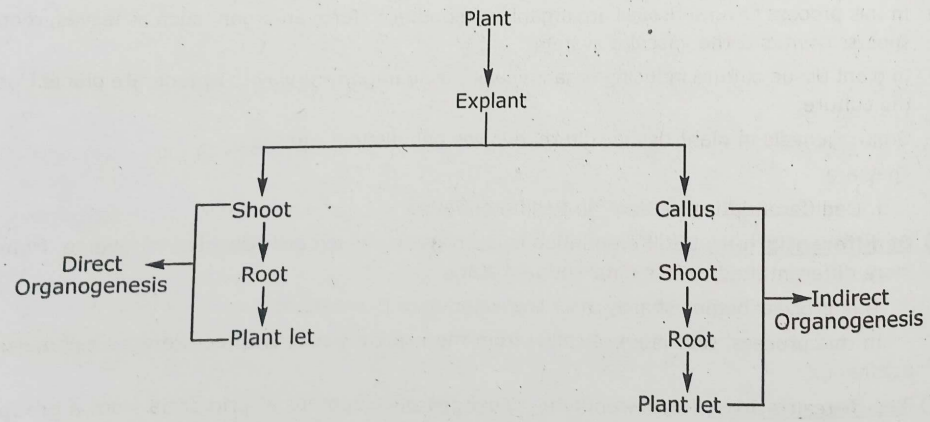
(i) Direct organogenesis :

In this direct organogenesis process callus formation does not occur. Hero directly explant developing shoot or root system (New Plant)

(ii) Indirect organogenesis :

In this indirect organogenesis process cell develops callus, here callus undergoes repeated divisions, to form root (or) shoot system (New plant)

Explant ⟶ Organ (Shoot / Root)



SOMATIC EMBRYOGENESIS

- Formation of embryo is known as embryogenesis.
- Somatic means – Somatic cells that is non-embryonic cells
- Formation of embryo from non embryonic cell i.e., vegetos is known as somatic embryogenesis.
- Somatic embryogenesis is an artificial process in which a plant or embryo is derived from somatic cell (Non–embryonic cell)
- Somatic embryos are formed from plant cells (Ordinary plant tissue) that are normally involved in the developement of embryos.
- No endosperm or seed coat is formed around a somatic embryo.
- The embryos formed by somatic embryogenesis (SEs) are called embryoids.
- Embryoid is a small well organised structure comparable to the zygotic embryo which is produced in vitro from non–zygotic cells.
- This process was discovered for the first time in daccus carota (carrot) by steward etal (1958) and Reinert (1959).
- Kohlenbach (1978) Classified the embryos in following manner.
  - i. Zygotic embryos - There are formed by fertilized egg or the zygote.
  - ii. Non – zygotic embryos - There are formed by cells other than zygote.
- Somatic embryogenesis (SEs) in vitro may be achieved through direct or indirect paths

I Factors effecting somatic embryo genesis:

- Explant is a source material to induce somatic embryogenesis (SES) are very diverse.
- There are very responsive plants. Such as carrot in which any part of the plant can be used to induce embryogenic cultures.
- Types of explants used in SEs are immature zygotic embryos or inflorescence, cell suspension culture, petioles or proto plants leaves, stems and also roots can be used for the somatic embryogenesis process.

II Plant growth regulators :

- Plant growth regulators are very important in plant tissue culture.
- Auxin, cytokinins, gibberellins play very important role in somatic embryogenesis.
  - i. **Auxin** - 2–4–DNAA has been the best synthetic auxin used for inducing somatic embryogenesis.
- Continuous supply of auxin causes embryogenic cells to divide with out the appearance of embryos.
- So embryonic cells after treatement with auxin must be transformed to auxin free medium that constitute the embryo developement.

ii. Cytokinins :

It produces globular embryo from initial embryos.

- High ratio of cytokinin than auxin induces shoot formation and reverse ratio (High ratio of auxins) favours.

iii. Gibberlins always inhibits the somatic imbryogenesis.

- ABA it is a inducing agent.
- ABA promote embryo maturation and prevent germination.

iv. Nitrogen source :

Reduced form of nitrogen is the sole source of embryo formation. Here nitrogen source is the veryimportant in somatic embryogenesis (SEs) and also polyamines, genetype and electrical stimulation are few factors which effect the somatic embryogenesis.

- Expose of explant to mild electric current of 0.02v DC for 20h promoted embryogenesis in alfalfa and Tobacco

Stages of somatic embryogenesis:

- Here somatic embryogenesis (SEs) always under go initially 3 stages.

  They are (i) Induction (ii) Development (iii) Maturation

I. Induction :

- An auxin particularly 2-4-D is generally necessary to induce embryogenesis.
- So requirements of exogenous auxin for induction of SEs depends on nature of explants. Used with relative concentration of auxin.
- It means that during the first stage. The initial requirement of auxin should be given to the explant.

II. Development :

- After reinitiation of cell division and a period of cell proliferation in presence of auxin.

  Embryogenesis cells are released in to auxin free medium.
- There cells are in the clusters of cytoplasmic cells called proembryonic mass of cells (PEMs)
- So after the treatment of auxin, the explant should be placed in to the media which is free from the auxin. Here the cluster of cells which are present in the media they are called pro embryonic mass of cells (PEMs)
- This PEMs cells will move next stage that is maturation it is a final stage.

III. Maturation :

- The quality of SEs with regard to their germinability or conversion into plants is very poor.
- This is because the apparently normal looking SEs are actually in complete in their developement.

  Unlike zygotic embryos, somatic embryogenesis do not go through the final phase of embryogenesis. Called embryo maturation. Which is characterized by accumulation of embryo specific reserve food materials and proteins which import desiccation tolerance to the embryos. Embryo size does not increase during this phase.
- Which prevent precacious germination and promotes normal development of embryogenesis by triggering expression of genes which normally express during drying down stage of seeds.
- Somatic embryogenesis achieved through direct or indirect paths.

1. Direct somatic embryogenesis :

- In direct path callus formation not occurs.

  Here callus formation is ABSENT.
- When embryos are formed directly from explant tissue creating an identical clone without production of intervening callus.
- The explants capable of direct embryo genesis same to carry competent or pre embryonic determined cells (PEDCs).
- Here explant may be Hypocotyl, endosperm, or Nucellus, these cells have pre embryonic determining cells (PEDCs).

- These cells are committed to embryo development.

2. Indirect somatic embryogenesis :

- Here callus formationis present.
- When explants produced undifferentiated mass of cells (Callus) which is maintained or differentiated in to embryo.
- Specific growth regulators and culture conditions are required for callus formation and the re determination of embryogenic development method called induced embryogenic determined cells (IEDEs).

Advantages of somatic embryogeneris:

1. It is observable, as its various culture conditions can be controlled.
2. Lack of material is not a limiting factor for experimentation.
3. High propagation rate.
4. Somaclonal variations.
5. Germplasm conservation.
6. Labour saving.
7. Elimination of diseases and viruses.
8. Synthetic seeds can be produced by encapsulating the SE's .

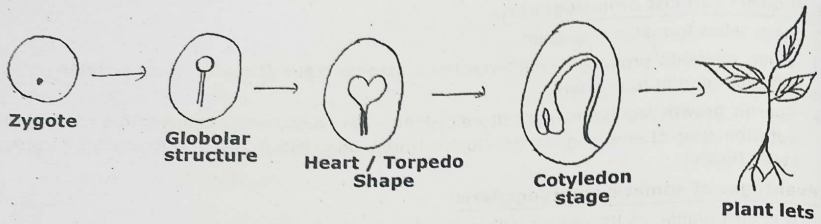
Disadvantages :

1. It confined to few species.
2. The somatic embryos show very poor germination because of their physiological and bio chemical immaturity.
3. Instability of cultured cells in long-term cultures is a major limitation in commericial exploitation and mass propagation of somatic embryogenesis.

ZYGOTIC EMBRYOGENESIS

- In Angiosperms the ovule contains a haploid egg cell or ovum which is a female gamete gets fertilised by the male gamete resulting in the formation of diploid zygote.

  This zygote gives rise to multicellular embryo which is known as zygotic embryo and the process must be called as zygotic embryogenesis.
- The zygotic embryo is formed following double fertilization of the ovule, forming the plant embryo and the endosperm which together go in to the seed.
- In this zygotic embryogenesis process embryos in vitro first develop to globular stage.
- The globular shape of embryo is lost, and it enters to heart shape stage.
- In this stage cotyledons (first leaves) begin to develop.
- Dicots have two catyledons which gives embryo a heart shaped appearance while in monocots such as maize, only a single cotyledon emerges.
- This process goes through the torpedo and plantlet stages and requires. 2.5 to 3 - weeks time.
- Zygotic embryos move in to the cotyledon stage & long maturation stage followed by desiccation and dormancy. It syntbersizes, Storage protein during maturation and desiccation dormancy is primarily regulated by abcisic acid (ABA).
- Later on it develops plant let.



SHORT ANSWER QUESTIONS

1. Preparation of culture medium
2. Sterilization of the medium
3. Micro propagation
4. Root culture
5. Shoot tip culture
6. Leaf culture
7. Anther culture (Pollen culture)
8. Ovule culture
9. Embryo culture
10. Protoplast culture
11. Callus culture
12. Organogenesis
13. Somatic embryogenesis
14. Zygotic embryogenesis

LONG ANSWER QUESTIONS

1. What is tissue culture? Write about the different stages in tissue culture.
2. What is micropropagation? Write about it?
3. Write about root culture, leaf culture, shoot tip culture.
4. Write about ovule culture, embryo culture.
5. What is callus culture? Explain the process and importance of callus culture.
6. Define and explain Organogenesis?
7. Explain somatic embryogenesis and Zygotic embryogenesis.

UNIT-II

APPLICATIONS OF TISSUE CULTURE

Tissue culture has several applications in the fields of agriculture, horticulture, forestry, pharmacology, medicine, environment and related industries. Some of the important applications are given below.

1) Production of pathogen free plants
2) Production of somaclonal variations.
3) Production of stress resistance plants
4) Production of secondary metabolites.
5) Synthetic seeds.

1. PRODUCTION OF PATHOGEN FREE PLANTS:

Generally plants show disease resistance characters naturally. The pathogenswhich cause diseases in plants are fungi, bacteria and virus. These pathogens inhibit the disease resistance capacity of plants by destroying plant tissues and express the disease symptoms. The diseases caused by the pathogens can be controlled by disease controllers. But the viruses are not controlled by the chemicals and thus kill the plants. Therefore, virus free plants can be produced through plant tissue culture.

Generally, meristematic tissue of a virus infected plant remains free from virus. Therefore, virus free plants can be produced through shbot tip culture of a virus infected plant.

Methods to prevent virus:

Following four methods are used to prevent viral diseases:

i) Heat treatment ii) shoot tip culture iii) Using chemicals iv) In vitro culture method

i) Heat treatment: Shoot tips or buds growing for the production of virus free plants are treating with 35 -40°C temperature by placing them in hot water or in hot air to kill virus. But most of the viruses show resistance to temperature. Plant tissue dies at high temperature. This method is used for only few plants.

ii) Shoot tip culture: Meristematic tissue of a health plant or virus infected plant remains free from virus. Therefore, Virus free plants can produce through the culture of shoot tip or meristem on suitable culture medium.

iii) Chemical treatment: Virus can be preventing by using few types chemicals in culture medium. Virus free plants can produce by reducing the concentration of cytokinins, auxins and the addition of antibiotics like thiouracil, acetyl salicylic acid in culture medium.

iv) In vitro method: Virus free plants can produce through other in vitro methods apart from the shoot tip or meristem culture. In which callus culture is most important one. In callus culture, pathogen free tissues are selected as explant and culture on medium. Identify the diseased areas in callus and select the virus free cells for culture.

2. Somaclonal variations:

The genetic variations found in the in vitro cultured cells are called as somaclonal variations. The plants derived from such cells are referred to as somaclones. These plants exhibit some advantages which are not found in parental plants. Useful traits like disease resistance, herbicide resistance and stress resistance can be seen in somaclonal variants. There are many factors to occur somaclones in culture. Mainly somaclones occur as a result of genetic heterogeneity (change in chromosome number and/or structure) in plant tissue culture.

The genetic changes associated with somaclonal aberrations like polyploidy, euploidy,

aneuploidy, translocations and inversions.

Isolation of Somaclones:

Two methods used to produce, isolate and selection of somaclones. They are:

i) Without in vitro selection. ii) With in vitro selection.

i) Without in vitro selection: Explant taken from the stem or leaf or root is cultured on suitable medium supplemented with growth regulators to produce callus. The unorganized callus sub cultured and transferred to shoot induction medium for regeneration of plants. The plants so produced are grown in pots under green house conditions, transferred to field and analyzed for somaclonal variants.

Somaclonal variants of several crops have been successfully obtained by this approach. Eg: potato, tomato.

ii) With in vitro selection: This method basically involves the handling of protoplast, callus in cultures like micro organisms and selection of mutants. Somaclones obtained from this method are resistant to toxins or inhibitory substances or survive under conditions of environmental stress.

The callus obtained from an explant is exposed in the medium to inhibitors like toxins, antibiotics or amino acid analogs. Selection cycles are carried out to isolate the tolerant callus cultures and these calli are regenerated into plants. The plants so obtained are tolerant against inhibitory substances.

Uses of somaclones:

1. Salt tolerant somaclones have been developed in Tobacco and Maize.
2. Seed quality is improved in the seeds of Lathyrus sativa.
3. In tomato quality fruits have been developed. These fruits contain high amount of solid state. These tomatoes are known as super- tomatoes.
4. Disease resistant somaclonal variants have been developed in many plants. Eg: paddy, wheat, apple, tomato etc. ..

3. Production of stress resistant plants:

Development of biotic and abiotic stress tolerant plants are of immense importance to increase food grains productivity. The plants growing in soils with high salt concentration are known as salt tolerant plants and the plants resistances to herbicides are known as herbicide tolerant plants.

a) Abiotic stress: The pH of the crop lands is different. Crop plants have to tolerate water stress and drought stress conditions. Salts in the soil like NaCl, CaCl, Na_2ZSO_4 etcetera can reduce the production efficiency of crop plants.

It is necessary to produce the crop plants having abiotic stress tolerant and high productivity.

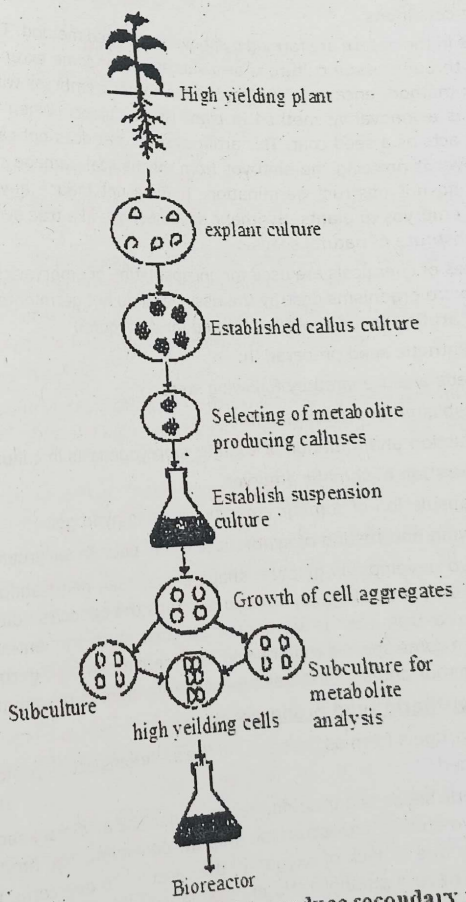
Salt tolerant Plants: Presence of high percentage of salts like NaCl, $CaCl_2$, Na_2SO_4, K_2SO_4 in the soils diminish the growth of crop plants. The plants which can grow in these conditions by withstanding high salt concentration are known as salt tolerant plants.

b) Biotic stress: Biotic stress is the stress caused by insects/pests which attack on plants due to environmental changes.

c) Herbicide Resistant Plants: Herbs (weeds) in the crop plants are destroyed by spraying glyphosate, atrazine etc. herbicides. These herbicides not only destroy the weeds but also kill the crop plants. Therefore, by altering the genes in crop plants can be protected from herbicides and increase in productivity. Herbicide resistant plants are produced by introducing herbicide resistant genes into the plants. Herbicides do not show any effect on herbicide resistant crop plants on spraying.

4. Production of secondary metabolites:

In general secondary metabolites are produced during plant cell culture. In which important secondary metabolites are alkaloids, glycosides, terpanoids, tannins etc. Secondary metabolites can be produced through tissue culture. There are some modern methods to produce high amounts of secondary metabolites. Firstly only those cells with high- yielding ability are selected and maintained by culturing. Single cell cloning and cell aggregate cloning methods are used in this process. A high yielding plant of the desired secondary metabolite is selected and its explants are first cultured on a solid medium. After establishing callus cultures, high metabolite producing calluses are identified and they are grown in suspension cultures. The freshly developed cell aggregates are divided into two parts. One half is grown further, while the other half is used for the quantitative analysis of the metabolite produced. The cell lines with high yield of secondary metabolites are culture in a bioreactor for large scale production.



Production of cells which produce secondary metabolites

Advantages:

1. In plant tissue culture cell growth can be controlled to facilitate improved product formation.
2. The quality of the product will be consistent as it is produced by specific cell line.
3. Recovery of the product will be easy.
4. Plant cultures are particularly useful in case of plants which are dificult or expensive to be grown in the fields.
5. Mutant cell lines can be developed for the production of novel chemical compounds.
6. Biotransforrnation reactions can be carried out to convert specific substance to voluble product.
7. The production time is less and labor costs are minimal.

5. SYNTHETIC SEEDS (or) ARTIFICIAL SEEDS:

We all know that ovules are modified into seeds after fertilization. Seed coat is formed around the seeds and gives protection to the seeds. The embryo in the seed germinates into seedlings under favorable conditions.

All plants in the nature are formed by above mentioned method. These days preparation of synthetic seeds through tissue culture is also successful for some extent. Production of somatic embryos in vitro method, encapsulation around the somatic embryos with chemicals to produce synthetic seeds is a innovative method in plant tissue culture. When chemicals are used for encapsulation, it acts as a seed coat. This artificial seed coat does not cause any damage to the embryos. More over it protects the embryos from mechanical damage while using embryos as synthetic seeds, do not obstruct germination, It does not induce any variations during the transformation of embryos to plants. Synthetic seeds behave like true seeds if grown in soil and can be used as substitute of natural seeds.

Various types of chemicals are used for encapsulation of embryos. If embryos not covered by artificial coat, micro organisms destroy the tissue and do not germinate in the soil. Therefore, by using hydrogels artificial seed coats are formed for protection.

Method of synthetic seed preparation:

Synthetic seeds are prepared by following steps.

1. Establishment of callus cultures
2. Induction and initiation of somatic embryogenesis in callus cultures
3. Maturation of somatic embryos.
4. Encapsulation of somatic embryos by using hydrogel.
5. Growing and testing of synthetic seeds in pots under green house conditions.

Initially, embryo develops as globular shape stage, then heart shaped stage and finally torpedo shaped stage. In the final stage, embryo develops the opposite poles for shoot and root development at the two extremities. In some plants species, such sequential developments may not be followed. Again in some species require cold treatment for embryo germination. Application of GA_3 also required for root and shoot development during embryo germination. eg. Citrus.

Chemicals used for synthetic seed preparation:

Water soluble hydrogels form as coat around the embrioids act as protecting capsule, thus synthetic seeds are formed.

Storage of synthetic seeds is a great limitation. When the artificial seeds are stored at low temperatures, the embryo show a characteristic drop in conversion. The limited storage time of artificial seeds is probably due to lack of oxygen in the capsule. To overcome this limitation, two possible solutions are, to have a smaller ratio of capsule volume to embryo volume so that gas diffusion can readily occur or to induce an arrested state in the emryo using growth control agent in the encapsulation medium.

Uses of Synthetic seeds:

1. Naturally seeds are produced in plants at the end of reproductive phase by the process of complex sexual reproduction. A plant may take long time for to attain reproductive phase. But artificial seeds can be produced within a month.
2. Plants produce the seeds at particular season of a year. But the production of artificial seed is not season dependent. At any season we may get artificial seeds.
3. Natural seeds remain in dormancy for a long period. By growing artificial seeds, this period may be reduced.
4. It is possible to produce the artificial seeds in economically important plants.
5. Preparation of synthetic seeds is one of the best methods to obtain genetically identical plants through clonal propagation. Eg. Santalum album.
6. Plants can be grown with less labor and expenditure by exporting the artificial seeds to different places.

HAIRY ROOTS

- Hairy root culture also called as transtormed root culture.
- This is produced after infection of plant tissues with the gram negative soil bacterium agrobacterium rhizogenes.
- These process takes places advantage of naturally occuring hairy root disease in dicotyledons.
- The disease is transformed by their genome. T-DNA from a bacterial plasmid to plant hairy root cells.
- Hairy root culture can be useful especially for the productions of root associated metabolites because of their high growth rate and genetic stability.
- Here a large number of small fine hairy roots covered with root hairs originated directly from the explant in response to Agrobacterium rhizogenes.

Induction of Hairy roots :

- When A Rhizogenes infect plant cell they transform the plant cell resultinting in the formation of the large number of small fine hairy roots covered with abondened root hairs and attempt hairy root.
- Agrobacterium rhizogenes is a soil borne gram negative bacterium and it contains a large roots inducing or ri-plasmid.
- When agrobacterium rhizogenes infects the plant it transfers a segment of DNA called T-DNA region from Ri-plasmid in to the genome of the infected plants.
- The T-DNA has 25-base pair direct oligonucleotide ripped and carries genes that code for auxin and cytokinin biosynthesis enzymes and genes for the synthesis of opines. Which are unusual amino acids, like octopine, agropine, nopaline, mannopine and cucumopine are used by A rhizogenes their sole source of carbon and nitrogen for further growth.
- T-DNA region contains at least four root loci/rol genes rol A, B, C, D first three is competent to induce hairy roots with faster frowth rafe.
- Different rol gene products affect plant growth and development in different ways.
- Agrobacterium infection is generally initiated by chemical signals. (Acetosyringone, a phenolic molecule) produced by the host plant that induce the activation of agrobacterium vinlence (vir) genes.
- Vir D_2 is a transpacific endonuclease that cut the T-DNA border ripped and attaches it self to one end of the leanous strand and the complex then exported to the host cell with the help of proteins encoded by the vir B oprone and the vir D_4 genes.

- Inside the host cell vir d_2 t – strand complex is coated with proteins encoded by vir E_2 and forms a mature t-complex which is then imported in to the host cell nucleus with the help of several bacterial and host protein.
- Inside the host cell nucleus the mature t-complex is uncoated by targeted proteolysis and final'y it integrated in to the plant genome.

Applications of hairy roots :-

- Functional analysis of genes.
- Expression foreign proteins.
- Production of secondary meltabolites.
- The culture may produce compounds which is not found untransformed roots.
- The culture may change the composition of metabolites.
- The culture can be used to regenerate, a whole polant.

Production of secondary metabolites by hairy root culture :

- Hairy roots produce a large variety of phyto chemicals and the range of metabolites produced represent all major classes of phyto chemicals. including alkaloids, coumarins, polyacetylenes, sesquiterpenes, napnthaquinones, tannins etc.,
- Many of these compounds are used as pharmaceuticals, insecticides etc.
- Hairy roots have been reported for well over 100 dicotyledonous species from a wide range of families like apocyunaceae. Arailaceae, Asteraceae, Boraginaceae, Chenopodiaceae, Compositae, cucurbitaceae, rubiaceae, solanaceae etc.....

L–Dopa : A precursor of catechola-mines, an important neurotransmitter used in treatment of of parkinson's disease.

Shikonin : Used as an anti bacterial and anti - ulcer agent.

Anthraquinone : Used for dyes and medicinal purpose.

Opiate alkaloids : Particularly codeine and morphine for medicinal purposes.

Berberine : an alkaloid with medicinal uses for cholera and bacterial dysentery.

Valepotriates : Used as sedatiue.

Ginsenosides : For medicinal purposes.

Rosmaric acid : for antiviral, suppression of endotoxin shock and other medicinal purposes.

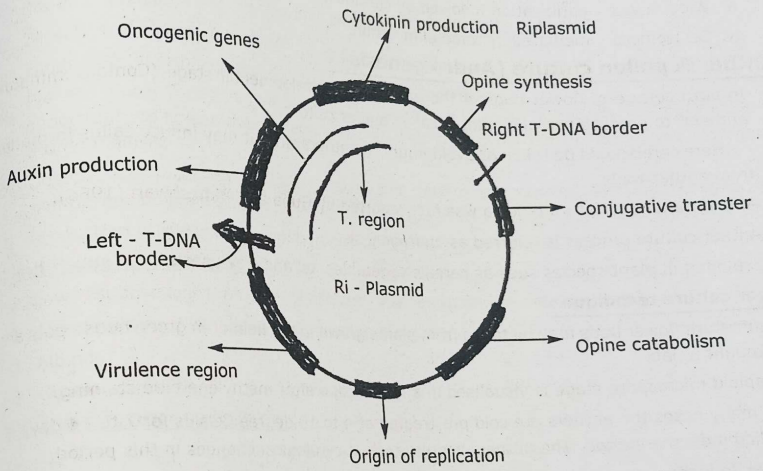
Quinine : Used for malaria.

Cardonolides : For treatment of heart disease.

- A selected list of plant species used in hairy root cultures for the production of secondary metabolites.

Plant species		Secondary metabolites.
Artemisia annua	–	Artemisinin
Atropa belladona	–	Tropane alkaloids, hyoscy - amine.
Betavulgaris	–	Betalin pigments.
Catharanthus roseus	–	Indole alkaloids
Datura sp	–	Tropane alkaloids
Nicotiana tabacum	–	Nicotine alkaloids
Solanum aviculare	–	Steroidal alkaloids
Solanum tuberosum	–	Steroidal alkaloids

Vinca minor	–	Vincamine
Withania somnifera	–	Withanolides.



HAPLOIDS

- Haploid plants containing one basic set of chromosomes (n).
- The presence of one set of chromosomes enables selection of mutants for specific traits.
- Development of haploids from hybrid plants shorten the breeding cycle in the development of novel in breds.
- Haploid production is ideal way to eliminate lethal genes.
- In nature, they arise as a result of parthenogenesis and there plants rarely produce the characteristics of male parent.

Gynogenesis : Production of haploid individual by the development of an unfertilized egg cell as a result of delayed pollination, at extremely low frequency through the process of parthenogenesis.

Ovule androgenesis : By the developments of the egg cell containing the male nucleus. Here egg cell nucleus is in activated.

- Artificial production of haploids was attempted through distant hybridization, delayed pollination, application of irradiated pollen, hormone treatments and temperature shocks – low frequency and not reliable.
- In vitro methods to produce haploids anther / pollen culture, ovary culture reliable.
- The development of vitro techniques for production of haploids was a major feat in the fields of plant biotechnology and plant breeding in the past few decades.
- Two Indian scientists Sipra Guha Mukherjee and S.C Maheshwari (1964) of Delhi university made the remarkable discovery of haploid plant production in angiosperms through Anther culture of 'Datura Innoxia'.

- Available methods of haploid plant production.
  - i. In vitro culture of anther (or) microspores
  - ii. In vitro culture of unfertilized ovary or female Gametophytic cells.
  - iii. Wide in vivo hybridization followed by chromosome elimination after fertilization.
  - iv. Centromere – mediated genome elimination.

Anther & pollen culture (Andro genesis) :

- In vitro culture of flower buds of the appropriate developmental stage (Contain immature anthers) to produce haploid plant is called anther culture.

  Here care should be taken to avoid injury to anthers since it may induce callus formation from anther walls.
- Anther culture of datura innoxia was first reported by Guha and Maheshwari (1964).
- Anther culture process is referred as androgenesis.
- Exploited in plant species such as cereals vegetables, oil and tree species.

Anther culture technique :

- Immature flower buds may be taken from plants grown in the field or in green house pots are brought to lab.
- Haploid microspore stage is visualised in a microscope after methylene blue staining.
- In many cases the anthers are cold pre-treated at 4 to 10 degree Celsius for 7 to 14 days is called inductive period. The anthers acquire certain cytological changes in this period.
- After inductive period the explants are surface sterilized, and the anthers are inoculated horizontally in the culture medium.
- In many cases anthers are cultured devoid of light for 3 weeks.

Pollen culture / microspore culture :

- In vitro culture of isolated pollen grains at microspore stage from early stages of flower bud is called pollen culture or microspore culture.
- Separation of pollen grains from flower buds
- After separation the debris are removed by gradient centrifugation or by filtration.
- Here pollen culture is advantageous over Anther culture.
- The anther culture has the disadvantage of regeneration occurs from diploid injured anther walls. This will not occur in pollen culture.
- Pollen culture is more efficient and also more convenient than anther culture.
- The tedious process of dissection of anthers is avoided in pollen culture. It is possible to macerate entire flower bud and separate the pollen grains by gradient centrifugation of filtration.
- Coming to the centrifugation process
- Wenzel (1975) introduced this method to isolate microspore from rye floral buds.
- The macerated pollen grains or suspension containing released pollen is layered on 30% sucrose solution and centrifuged at 1200g force for 5 min.
- Pollen grains are separated from band using the micropipette and cultured on culture medium, later cultures incubated for 3-4 weeks. After incubation it produces haploid plant lets.

Applications of Anther / pollen culture :

1. Production of stable, homozygous dihaploids or doubled haploids (DH) in a single generation by doubling the chromosomes either spontaneously (Eg-Rice) with chemical treatment like colchicine (Eg. Maize).
2. Doubled haploids (DH) considerably shortens the breeding cycle.
3. Detection of recessive mutants.
4. Elimination of lethal genes.
5. Gameto clonal variation : variation occurs during in vitro androgenesis provides, a unique opportunity to screen the gametophytic variation caused by recombination and segregation during meiosis.
6. Detection of recessive haploid mutants and rapid obtainment of the mutated gene in a homozygous diploid state.
7. Production of super male in Asparagus officinalis.

Limitations of anther culture:

- Low response less than 10% of anthers only responded to androgenesis.
- Abnormalities in embryogenesis mediated regeneration.
- Albinos
- Genetic instability

Pollen cryopreservation :

- Coming to the conservation & management of haploid genetic diversity through pollen cryopreservation.
- Here genetic diversity is one of the foremost pre requisites for any crop improvement programme.

  Pollen which is a product of genetic recombination has enormous potential to release diversity at the (Haploid) gametophytic stage.
- Attempts to conserve pollen at sub-zero temperatures (-10 to -20^{0}C) resulted in low viability associated with reduced fertility levels after prolonged durations of storage.
- Successful cryogenic preservation of pollen (In liquid nitrogen at -196^{0}C) has been accomplished in about 30 species of Agri horticultural importance at IIHR, (Indian Institute of Horti-cultural Research) without much sacrifice on viability and fertility profiles.
- Gene pool cryopreservation in the form of pollen could lead to establishment of pollen cryobanks serving as male gametophytic inputs for breeding new cultivar genotypes having high agricultural valve, by way of broadening the genetic base.

TRIPLOIDS

- A cell is called triploid when it has three complete sets of chromosomes, rather than the typical pair of chromosomes, it is referred as '3n' or (3x).
- Triploid plants are sterile, because chromosomes need to occur in pairs to produce viable offspring.
- The plant will still flower, but these flowers cannot be fertilized, because the gametes, or pollen and ovules, have a chromosomal number three that won't match up with the chromo.
- Somal number two of fertile gametes. The plants can still bear fruit, but the fruit is sterile, such as a seedless watermen, Banana etc.

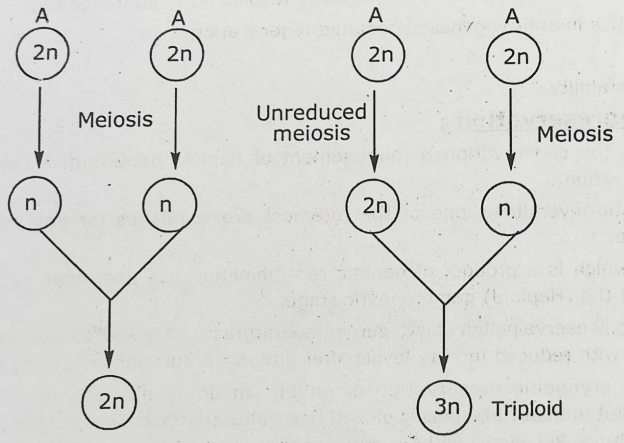
- Triploid plants are most useful in crop improvement where seed is not economic part.
- Triploids are exploited crops like Banana, Apple, Watermelon, Tea, Sugar beet, Mallberry Acacia, etc..,
- Here triploid plants have characters such as larger organs like leaves, fruits, flowers etc., greater vigor, higher biomass and stress resistance etc...

Methods of triploid production :

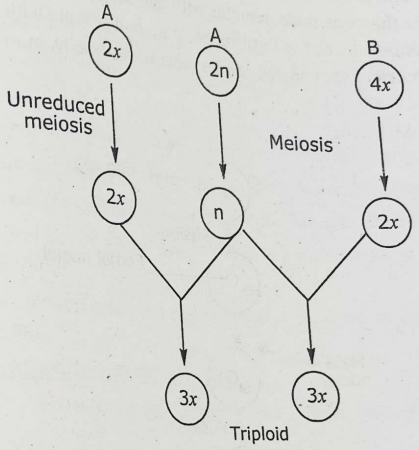
1. Natural selection
2. Artificial hybridization
3. Endosperm culture in vitro

1. Natural selection :

- Here fertilization occur unreduced gamete ($2x$) with the normal gamete (n) and form triploids.
- It is rare in nature because of inviable seeds and hence no progeny. It can be identified by faster growth and seedless in nature.

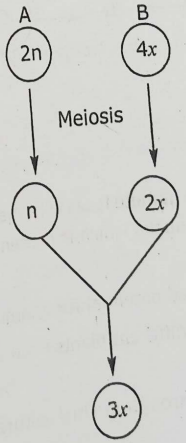


- Here environmental stress variables many stimulate production of unreduced gametes in diploids.
- Fertilization of the gametes from two species of different ploidy level.
- It result in increase in size of somatic cells & no.of chloroplast.
- Natural triploids naturally exist in nature like Populus termula, Populus alba, Quercus SPs, miscanthus SPs, etc...,

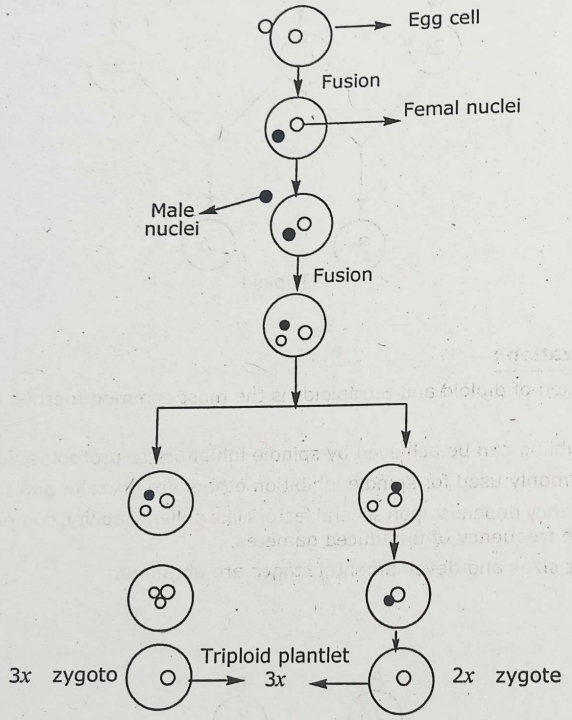


2. Artificial Hybridization :

- Sexual hybridization of diploid and tetraploids is the most common method i.e $2x$ x $4x$ or $4x$ x $2x$.
- Chromosome doubling can be achieved by spindle inhibitors or protoplast fusion.
- Colchicine is commonly used for spindle inhibition others are oryzalin and trifluralin.
- Here triploid efficiency depends upon several factors like pollen viability, compatibility between the parents or the frequency of unreduced gametes.
- Seeds of different sizes and developmental stages are obtained.



- Polyspermy is also one of the artificial hybridization.
- Fertilization of more than one male gametes with egg cell is known as polyspermy.
- Polyspermy block usually block the fertilization of second gamete with the fertilized egg.
- Fertilization of two nuclei with egg cell can be induced artificially, in invitro conditions.



Endosperm culture :

Endosperm culture methods :

- During fertilization process, one male gamete fuses with the egg cell and forms diploid zygote, and 2nd male gamete fuses with two nuclei which is present in centre of the embryo sac and forms triploid endosperm.
- Endosperm cells are totipotent and has potential for unlimited growth.
- Amount of endosperm varies among different plants from very little (legumes or cucurbits) to huge amount (cereals).
- Triploids can be directly regenerated through in vitro culture of endosperm tissue.

- Here 2 types of endosperm culture's are present
  1. Direct regeneration (without callus phase)
  2. Indirect regeneration (Callus formation occurs).
- Most direct and efficient method of triploid production.

Challenges :

- Mixoploid chimera appear frequently in endosperm culture.
- Production of aneuploids have also been reported from endosperm culture.
- Cells with different ploidy may respond differently towards different plant hormones.
- Induction of morphogenic responses or shoot regeneration from endosperm derived callus has low success rate.
- In several cases complex media are used for in vitro culture of endosperm. Which compromises the reproducibility.

Factor affecting endosperm culture :

i. Genotype ii. Developmental stage iii. Culture medium.

i. Genotype :

- Endosperm response on the in vitro culture is genotype dependent.
- Triploid plant lets have been regenerated from only a few plant species.

ii. Developmental stage :

- Age of endosperm is critical however it varies among various plants maize mature endosperm in Jatropha, Ricinus etc.
- Initial association of the mature endosperm with embryo is critical for induction.
- Germinating embryos may contribute factors to induce cell division.

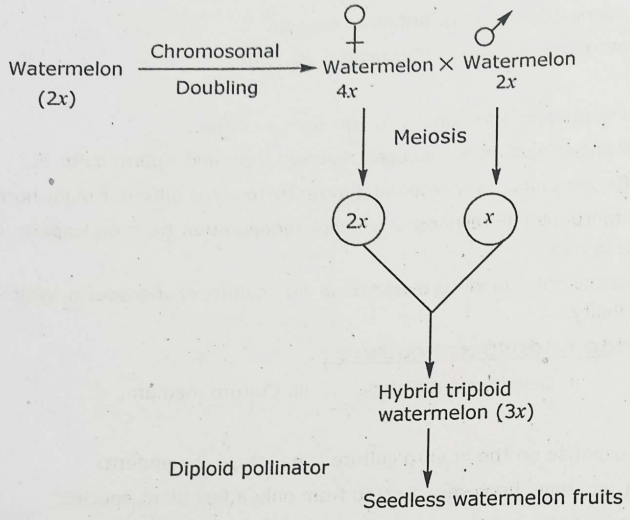
iii. Culture medium :

- Ms basal medium is commonly used with other supplements.
- Complex media like potato extract, tomato Juice, Corn juice, or yeast extract have been used successfully for endosperm culture.
- Use of auxins or cytokinin is required for endosperm cultures (2, 4-0 is more successful auxin).
- Supplementation of media with organic nitrogen is essential like casein hydrolysate.
- For regeneration of shoots from callus hormone supplements are essential.

Applications of triploids

Seed less ness :

- Triploids are sterile, and do not make seeds.
- Seedless fruits are desirable like grapes, banana, watermelon etc.....



Prolonged flowering :

- Flower are generally large and more colorful, and are desirable for commercial interests.
- Flowers have longer shelf – life, hence have better performance and give higher returns.

Photosynthetic efficiency :

- Larger somatic and guard cells.
- Higher number of chloroplasts.
- Better photosynthetic efficiency that result in vigorous growth and accumulation of higher biomass.

Neutralizing invasiveness :

- Introduction of plant of commercial interests to new regions.
- Invasive plants usually produce large amount of seeds.
- Triploidy can be used to control invasiveness of these species.
- Triploids may have better nutritive value like protein content of triploid mulberry is about 4% higher than diploid mulberry.
- Triploids are useful to study genome evolution, imbalance in gene dosage and genomic rearrangements.

CRYOPRESERVATION

- Cryopreservation is the process of cooling and storing cells, tissues, or organs at very low temperature below the freezing point (-196°C) to maintain their viability.
- In Greek krayos – means – frost.

- Cryopreservation is a technique of pressuring the plant cells in the frozen state using 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.
- The principle involved in cryopreservation is to bring the plant cell and tissue cultures to a zero metabolism or non dividing state by reducing the temperature in the presence of cryoprotectants.
- The cryopreservation technique involves following steps.
  1. Selection of material
  2. Addition of cryoprotectant
  3. Freezing
  4. Storage in liquid nitrogen
  5. Thawing
  6. Washing and re-culturing
  7. Measurement of viability
  8. Regeneration of plants.

1. Selection of plant material :

- Wide range of plant materials have been preserved by employing cryopreservation method.
- Suitable plant materials are selected from different plant species for cryopreservation examples meristem (potato), Root tip (Potato) Pollen (Mustard, rape), pollen embryo, zygotic embryo (wheat, rice) fused protoplasts (Pea x wheat) protoplasts (Datura), callus (Sugarcane), cell suspension (Datura) Among these, meristematic cells and suspension cell culture in the late lag phaseor log phase are most suitable.

2. Addition of cryoprotectants in pre-treatment :

- Cryoprotectants are the compounds that can prevent the damage caused to cells by freezing or thawing. The freezing point and supercooling 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.

3. Freezing :

- The sensitivity of the cells to low temperature is variable and largely depends on the plant species 4 different types of freezing methods are used :

a. Slow cooling slow thawing :

- Freezing method – the tissue or plant material is slowly frozen at slow cooling rate (0.5-2 c/ min) and thawing is done at a rate < than 25°C. The advantage is the plant cells are partially dehydrated and survive better.

b. Rapid cooling rapid thawing :

- It involves plunging the vials in liquid nitrogen (-196°C) and thawing is done rapidly 200-500c/min to prevent recrystallization.

c. Dry freezing method : In this method dehydrated cells and seeds are stored.

d. Vitrification : Modern technique based on sudden cooling of cells at temperature of -196°C in seconds and thawing is done at slower rate. High doses of cryoprotectants are used to avoid cell damage.

4. Storage : The maintenance of the frozen cells or material at specific temperature is very important. In general the temperature is kept -70 to -196 degree prolong storage is done at temperature of -196 degree c in liquid nitrogen. To prevent damage, continuous supply of nitrogen is done.

5. **Thawing :** Usually carried out by plunging the vials in to warm water bath with vigorous swirling. As thawing occurs the vials are transferred to another bath at 0 degree.
6. **Re-culture :** The preserved material is washed few times to remove the cryoprotectant. This material is then recultured in a fresh medium.
7. **Measurement of survival / viability.**

- There is possibility of death or cells due to storage stress.
Thus viability can be found at any stage. It is calculated by formula.

$$\frac{\text{No.of cells / organs growing}}{\text{No.of cells / organs thawed}} \times 100$$

8. **Plant regeneration :** The viable seeds are cultured on growth medium suitable environmental conditions are maintained and ultimately desired plant is regenerated.

Achievements :

1. Meristems of potato, cassava, sugar cane, etc... are stored for 2-4 years. The viability of cryopreserved meristems is 20-42 %.
2. Cell suspensions of soyabean, carrot, datura etc... are stored for 2-4 years. The viability is up to 50%.
3. Embryos of black gram, green gram, wheat, rice, coconut, etc... are stored for a long time. The viability is up to 55%.
4. Pollen grains of peanut, mustard, cotton, wheat, rice, belladona, etc... are stored for a long time.
5. Fused protoplasts of wheat, rice, etc... can also be stored in this method.

Advantages of cryopreservation :

1. Cryopreservation offers a possibility to preserve original genetic stocks in a limited area.
2. It ensures genetic stability in cryo-reserved materials.
3. It protects the viability of recalcitrant seeds in which embryos are short living due to impacts of the climate.
4. It can be used to store rare species.
5. It enhances the longevity of cryopreserved pollen, embryos and other plant materials.

Germplasm conservation :

- Germplasm is a living tissue (total content of genes / hereditary material), which can be conserved for long periods and whole plant is regenerated whenever it is required in the future.
- Germplasm conservation is the preservation of the genetic diversity of the plant or the genetic stock for its future use.
- It is necessary to preserve endangered plants or else some of the valuable genetic traits.
- Germplasm conservation is the most successful method to conserve the genetic traits of endangered and commercially valuable species.
- IBPGR (International Board of Plant Genetic resources) provide necessary support for collection, conservation and utilization of plant genetic resources through out the world.

Types of germplasm preservation

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

& Invitro conservation

i. Cryopreservation
ii. Cold storage
iii. Low pressure and low oxygen storage

B. In vivo conservation.

1. In – situ conservation

- The germplasm is conserved in natural environment in biosphere reserves such as national parks, sanctuaries.
- Preservation of native land plants in a near natural habitat along with several wild types.

2. Ex-situ conservation

- Used to preserve germplasm obtained from cultivated and wild plant materials.
- The genetic material in the form of seeds or in vitro cultures are preserved and stored as gene banks for long – term use.

(A) invitro conservation :

- Here gene banks have been made to preserve the genetic resources by non conventional methods such as cell and tissue culture methods.
- This will ensure the availability of valuable germplasm to breeder to develop new and improved varieties.
- Under the vitro conditions three methods are followed to preserve for long time storage (i) cryopreservation (ii) cold storage and (iii) Low pressure and low oxygen storage methods are used to preserve germplasm.

(i). Cryopreservation :

- The germplasm is stored at a very low temperature
- Generally the solid carbondioxide (at-79°C). Deep freezers (-80°C), vapor nitrogen (-150°C) and liquid nitrogen (-196°C) storage are used to maintain low temperature.
- The plant cell under go complete in active state, and can be conserved for long periods.
- Any tissue from a plant can be cryopreserved. Ex. Meristems, embryos, endosperms, ovules, seeds, cultured plant cells, protoplasts, calluses.
- Here cryoprotectants are used to preserve.
- They are such as DMSO preserve (Dimethyl sulfoxide), glycerol, ethylene, propylene, sucrose, mannose, glucose, proline, acetamide are added during the cryopreservation.

(ii) Cold storage :

- It is a slow growth germplasm conservation method at a non freezing low temperature (1-9°C).
- The growth of the plant material is slowed down in cold storage, and thus prevents cryogenic injuries.
- Several grape plants have been stored for over 15 years by using a cold storage at temperature around 9°C and transferring them in the fresh medium every year.
- Strawberry plants could be preserved at 10°C for 6 years.

(iii) Low pressure and low oxygen storage :

- The atmospheric pressure surrounding the plant material is reduced in low pressure storage.
- Low pressure reduces the in vitro growth.
- The oxygen concentration is reduced in the low oxygen storage.

- In low oxygen storage, the oxygen concentration is reduced and the partial pressure of oxygen below 50mmHg reduces plant tissue growth.

(B) In vivo Ex-situ conservation :

- Grown in an artificial environment like research institute germplasm plots, green house, glass house, etc...
- This will ensure the availability of germplasm for crop improvement.

SOMATIC HYBRIDIZATION

The process of production of a hybrid plant by fusing the protoplasts of two distinct species is called somatic hybridization. First somatic hybrid was developed by Peter Carlson and his colleagues in tobacco. They called this process as **parasexual hybridization.**

Procedure: there are three phases in somatic hybridization. They are

1. Protoplast Isolation. 2. Protoplast fusion. 3. Selection of hybrid cells.

1. Protoplast Isolation: Protplasts are isolated from the cells either through mechanical method or enzymatic method. Protoplasts are isolated from roots, stem, leaves, meristematic tissues, pollen grains, callus tissue etc.

a) Isolation of protoplasts from Leaf: fully expanded leaves of a young plant are selected, these leaves are placed in 70% ethyl alcohol for one minute later 20 minutes in 2% sodium hypochlorite. After that the leaves are washed with distilled water. Later on hypodermis of the leaf is removed and cut into small pieces. These pieces are placed in petridishes containing enzyme mixture (0.5% mecerozyme + 2% cellulose+13% sorbitol, pH=5.4). These petriplates are incubated at 25°C for 15 to 20 hours. After that enzyme mixtures filtered to separate protoplasts from leaf residue. Isolated protoplasts are purified with 13% sorbitol solution. Finally protoplasts are purified with 20% sucrose solution and centrifuged at 200rpm. As a result viable protoplasts are floated on the solution. Floated protoplasts are separated with a pipette.

b) Isolation protoplasts from pollen grains: pollen grains are separated from mature anthers by speedy shaking. The separated pollen grains purified by placing them in 2% sodium hypochloride for 10 minutes and centrifuged at 300 rpm for 10 minutes. Floated pollen grains are removed and purified with pure water. Then pollen grains treated with enzyme mixture(2% cellulose, 1% mecerozyme, 0.5% hemicellulase, 15% sucrose) as a result protoplasts are released from pollen grains.

c) Isolation of protoplasts from callus tissue: More number of protoplasts can be isolated from actively growing callus cells. Such cells are treated with 2-4% cellulose enzyme, mannitol and placed in hot water bath shaker for 4-6 hours at 30°-33°C. As a result protoplasts are released from callus cells.

2. Protoplast Fusion: Isolated protoplasts are fused in the presence of poly ethylene glycol (PEG). Protoplasts of two distinct species can be fused. Protoplast fusion can occur in two ways. They are.

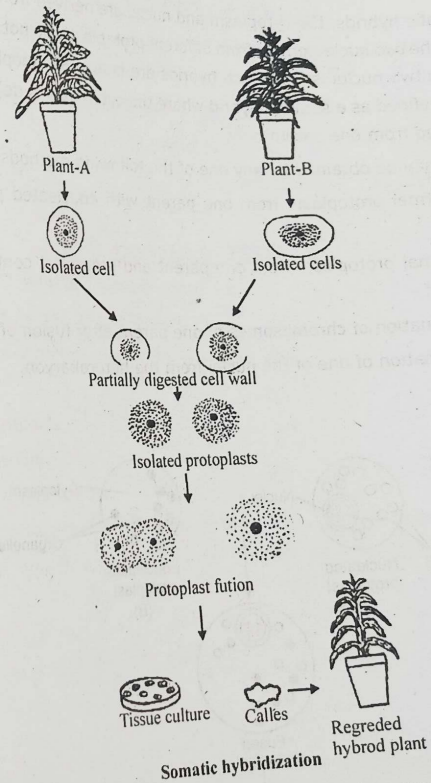
a) Spontaneous fusion b) Induced fusion.

a) Spontaneous Fusion: protoplasts may be fused when there close to each other during isolation. This type fusion is called spontaneous fusion. Spontaneous fusion takes place in between the protoplasts of same species.

b) Induce fusion: it is the fusion induced to fuse the protoplasts of two distinct species. The chemical agents used to fuse the protoplasts are called fusogens. Sodium nitrite, poly ethylene

col, poly Vinyl alcohol, dextrons are used as fusogens in plants.

There are three main stages in the protoplast fusion. In the first stage protoplasts are me closer to each other. In the second stage protoplasts membranes get fused at localized nts of adhesion. This leads to the formation of cytoplasmic bridges between protoplasts. In rd stage the fused protoplasts get rounded as a result of cytoplasmic. bridges leading to the rmation of spherical homokaryon or heterokaryon.



Somatic hybridization

3. Selection of hybrid cells: after the protoplasts fusion homokaryons or heterokaryons are formed. There are about 0.5% to 10% homokaryons in the total populations. Heterokaryons are not so profitable. It is therefore necessary to select the hybrid cells and grow somatic hybrids from these cells. Markers are used to recognize the heterokaryons. For example green protoplasts of tobacco and red anthocynin of carrot can be used as markers to identify the somatic hybrid cell between tobacco and carrot

Advantages:

1. Genes that determine the characteristic features of disease resistance, insect resistance, drought resistance, cytoplasmic male sterility can be introduced into crop plants by using somatic hybrids in genetic transformation experiments.

2. Somatic hybrids can be developed through somatic hybridization in sexually incompatible species.
3. Disease resistant traits are found in wild varities. These disease resistant traits can be introduced into crop plants through somatic hybridization.

CYBRIDS (CYTOPLASMIC HYBRIDS)

In somatic hybrids, the cytoplasm and nuclei are derived from both the parents. However in most cases, the two nuclei coming from different protoplasts, do not fuse and genetic information from one of the two nuclei is lost. Such hybrids are known as cytoplasmic hybrids or cybrids. A cybrid may be defined as a somatic hybrid where the cytoplasm is derived from both parents and nucleus is derived from one parent only.

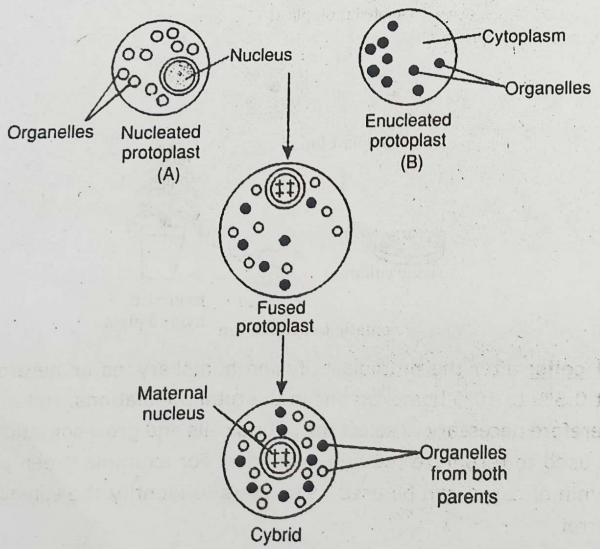
Cybrids may be obtained by any one of the following methods :

(a) Fusion of normal protoplasts from one parent with enucleated protoplasts from the other parent.

(b) Fusion of normal protoplasts from one parent and protoplast containing non viable nucleus from the other.

(c) Selective elimination of chromosomes of one parent after fusion of the nuclei and

(d) Selective elimination of one of the nuclei from the heterokaryon.



Formation of a cybrid due to fusion of normal protoplast with a enucleated protoplast

Applications:

1. Streptomycin resistant character in Nicotiana tobaccum transferred to Nicotiana silvestris through cybrids production.
2. More nectar secreting varieties in Brassica were developed through cybrids production.

SHORT ANSWER QUESTIONS

1. Production of pathogen free plants
2. Somaclonal variations
3. Production of stress resistant plant
4. Synthetic seeds
5. Hairy roots applications
6. Production of secondary metabolities by hairy root culture
7. Haploids
8. Triploids
9. Cryopreservation
10. Germplasm conservation
11. Somatic hybridization
12. Cybrids

LONG ANSWER QUESTIONS

1. What is tissue culture? Mention its application.
2. Production of Hairy roots and its applications.
3. Production of secondary metabolites by hairy root culture.
4. Explain Haploids.
5. Define and explain triploids.
6. Explain cryopreservation.
7. Explain germplasm conservation.
8. What is somatic hybridization? Write about its procedure, applications.
9. What is cytoplasmic hybridization (or) Cybrids? Mention its procedure, applications.

UNIT-III

BIOTECHNOLOGY

Biotechnology is the technology of utilizing micro organisms at industrial level for generating useful products essential to human welfare. It is also called as Genetic Engineering.

A wide variety of materials and services that are useful to humans. It's obtained from microbes is known as bio technology and it is also known as genetic engineering.

History :

The origin and development of biotechnology can be seen under three subsections. They are

(a) Ancient Biotechnology

(b) Traditional Biotechnology

(c) Modern Biotechnology

a. Ancient Biotechnology :

The one that existed 1800 years ago is called "Ancien Biotechnology"

This includes the production of alcoholic

- This includes the production of alcohols beverages, vinegar, etc... by fermentation and yeast is widely used in the fermentation process.
- This first biotechnology product was cheese. It produces rennet from milk through an enzyme using microorganisms.
- In BC 7000 years. In china they used yeast in beer and wine production. Approximately BC 6000 years most people in India produce curd and cheese using bacteria that produce lactic acid.

b. Traditional Biotechnology :

It stretched from 1880 to 1952. Since the definition of biotechnology, some of the discoveries have been made using organisms, bacteria, plants, and animals.

c. Modern Biotechnology :

After discovery of DNA the modern Biotechnology was emerged.

Scope :

The goal of bio technology is very broad because it makes products related to health, agriculture, industry, and the environment

APPLICATIONS OF BIOTECHNOLOGY

(OR)

ROLE OF BIOTECHNOLOGY IN DIFFERENT FIELDS

Following important fields are influenced by biotechnology.

I. ROLE OF BIOTECHNOLOGY IN AGRICULTURE:

1) Nitrogen fixing Microorganisms: Nitrogen is the important nutrient for plant growth. However, 78% of nitrogen is present in the atmosphere, higher plants can't absorb. Only few types of microorganisms can fix this molecular nitrogen and converted into ammonia (NH_3). These microorganisms are known as nitrogen fixing organisms. Eg. Rhizobium, Azatobacter, Acetobacter, Klebsiella like bacteria and Anbena, Nostoc like blue green algae.

In these organisms nitrogen fixation is under control of genes. These genes are called as nif genes. By the development of r-DNA technology nif genes can be isolated from the Rhizobium species and transferred to other plants.

2) Phosphorus soluble Microorganisms: Phosphorus plays a major role after nitrogen in plant nutrition. Plants can absorb this element in phosphate form. In most of the soils phosphate is in the form of water insoluble rock phosphate. So plants cannot absorb this phosphorus. Some types of microorganisms can dissolve this phosphate and make the plants absorb. These microorganisms are called as phosphate solubilizers. Phosphate deficiency in the soil can correct by growing the micro organisms. Thus the use of fertilizers will decrease. Eg. Pseudomonas, Bacillus like bacteria and Penicillium, Fusarium, Aspergillus like fungi.

3) Bioinsecticides: Various types of microorganisms are parasites on insects/pests and prevent their growth.These are called as entamopathogens. These microorganisms are produced in large scale by culture, used as insecticides by spraying on crop plants. Examples: Bacillus thuringenesis, Baspericus, Hirsutella thamsonai, Nuclear polyhedrosis virus. The bacteria Bacillus thuringenesis prepare toxic protein (Cry-protein) while producing spores. This cry-protein destroys insect larvae. Thus the gene bt-2 isolated from the bacteria cell and inserted into"tomato, tobacco, and cotton plants by Agrobacterium Ti-plasmid. Because of this the growth of insect larvae prevented by the cry-protein formed in these plants.

4) Production of disease resistant plants: Through r-DNA technology, desired genes can be introduced into crop plants to improve resistance against diseases, insecticide substances, and adverse environmental conditions. Herbicide resistant genes are isolated from Salmonella typhi, Escherchia coli and introduced into tobacco, tomato plants. Similarly some herbicide resistant genes are isolated from Medocago sativa and Amaranthus hybridus, introduced into wheat, tobacco plants. Crop plants tolerant to adverse environmental conditions can be produced by genetic transformation.

5) Production of nutrient rich crop plants: Food materials are stored in the form of protein in grains and pulses. The amino acid lysine is present in low amounts in grains.Similarly the deficiency of sulphur containing amino acids is more in legumes. The plants which produce high amounts of amino acids can be developed and isolated through somaclones.

II) ROLE OF BIOTECHNOLOGY IN MEDICINE:

Various substances which improve disease resistance, substances which control diseases in human beings and animals and methods useful for diagnosis have been developed through"biotechnology.

1. Vaccines:

The proteins produced by pathogenic microorganisms act as toxic, substances and cause diseases. In high disease resistant organisms a different type of proteins are produced to reduce the activity of antigen proteins. These proteins are known as antibodies. This type of immunity is known as acquired immunity. Edward Jenner first reported that, immunity in humans can be improved by vaccines. Generally vaccines are prepared from the antibodies isolated from the diseased animal bodies. Genes which produce antibodies are isolated and introduced into bacterium cells through r-DNA technology. These genetically modified bacteria acquired pathogenic characters. These bacteria cells are collected and used as vaccines. Vaccines which are produced by this method are used to prevent the diseases like typhoid, cholera, malaria, cancer, rabies etc.

2. Interferons:

A kind of glycoprotein produced by virus infected cells prevents virus replication. These special proteins are called as Interferons. Gilbert and Wiseman (1980) reported that, these proteins can be produced in E. coli cells through r-DNA technology. By using these interferons immunity

can be developed against different diseases in humans caused by viruses. Leucocytic interferons, α -interferons are used for the treatment of breast cancer~and renal carcinoma respectively.

3) Hormones:

Different types of hormones are produced through biotechnology. Insulin is produced in the pancreas of humans. Humans get diabetic disease when this hormone is not produced insuficient quantities. Thus insulin is given in the form injection for these patients. A few years ago, this hormone was collected from livestock and pigs. When insulin producing genes introduced into bacterium cells, these genetically modified bacteria start producing insulin hormone which is useful to human beings. This hormone is marketing in the name of humulin.

4) Disease Control:

Gene therapy is used to cure genetic disorders that occur in human beings. In this gene therapy the abnormal gene that causes disease have been identified and replaced by normal gene."Currently there is no significant progress in this regard.

III) ROLE OF BIOTECHNOLOGY IN INDUSTRY:

1) Advantages of Fermentation:

Many valuble and useful chemicals are produced by using microorganisms through fermentation at industrial level. Fermentation occurs with the help of anaerobic enzymes. In this process complex organic material are converted into simpler form. Chemicals like amino acids, enzymes, proteins, organic acids, antibodies, single cell proteins (SCP) are produced through this process.

2) Production of Antibiotics:

The genus Streptomyces is the source for the most of antibiotics. Antibiotics are also produced by Penicillium, Nocardia, few Actinomycetes species, Cephalosporium species.

3) Alcoholic beverages:

Alcoholic beverages like beer, brandy, whisky, wine-etc can be produced by fermentation process.

IV) Role of biotechnology in the field of Environment:

Biotechnology techniques are used to control pollution and waste treatment. Biotechnology methods have been identified as more efficient than conventional methods in waste treatment. For this, environmental engineers followed bioremediation procedure. Introduction of nutrients into polluted soils promote the growth of bacterial microorganisms or bacteria are newly introduced into this type of soils. These bacteria react and break down the waste materials. Thus, these waste materials modified into simpler and non toxic end products.

V) Role of biotechnology in shrimp industry and Aquaculture:

The following useful aspects are achieved in this filed through biotechnology.

1. Lupines, chick pea plants which contain more Omega 3-fatty acids modified as feed useful for fishes.
2. Large and early breeding varities of Shrimps (prawns) etc have been developed.
3. Valuble products useful for aquaculture have been produced from wastage of domestic animals.

VI) Role of Biotechnology in mining:

1. Biological processes or methods used for isolation and collection of metals from the mines.This is called as biomining.
2. Efforts are going to improve coal purification processes and changed it into chemically usable energy. This is known as coal bioprocessing.

RECOMBINANT — DNA TECHNOLOGY

It is a technique used in genetic engineering. This technology involves identification, isolation and insertion of gene of interest into a vector and such hybrid vector is introduced into a host cell. This technology was discovered by Cohen and Boyer. The organism that has this r-DNA is called recombinant organism. Eg. The gene responsible for insulin production was isolated from human cells and transferred to the bacterium E. coli. This genetically engineered E.coli produces human insulin (Humulin).

This technology has four steps. They are

I) Selection and isolation of gene (DNA) of interest.

II) Introduction of DNA-insert into vector.

III) Introduction of recombinant vector into a suitable host.

IV) Selection and screening of transformed host cells.

1. Selection and isolation of gene (DNA) of interest:

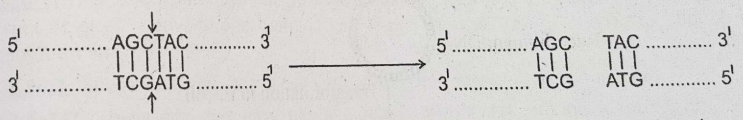
Cell wall is digested by using enzymes.

- Detergents are used to dissolve cell membrane.
- DNA is isolated from protoplasm using phenol or some nuclease enzymes.
- Further it is subjected to gradient centrifugation to purify the DNA.
- DNA cut into fragments using restriction endonucleases. Restriction enzymes were discovered by Arber, Smith and Nathans for the first time in bacterium cell.

Restriction enzymes cut the DNA molecule in two ways. They are

a) Blunt ends. b) Sticky ends.

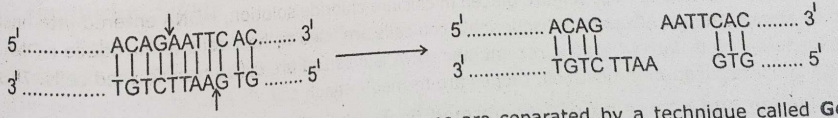
a) Blunt Ends: Some restriction enzymes recognize and cut the DNA strands at precisely opposite points. Thus non polar ends of DNA fragments are formed. These ends are called as blunt ends.



b) Sticky ends: But most of the restriction enzymes not cut the DNA strands directly opposite each other, instead the cuts are staggered forming short complimentary single stranded sticky ends due to the presence of palindromic sequences.

The restriction enzyme EcoRI recognizes GAA sites and cut DNA between G and A (G ↓ A).

Thus sticky ends are formed.



The resultant fragments of restriction enzymes are separated by a technique called **Gel Electrophoresis.**

Electrophoresis: The desired fragments are selected and isolated by a technique called Southern blotting.

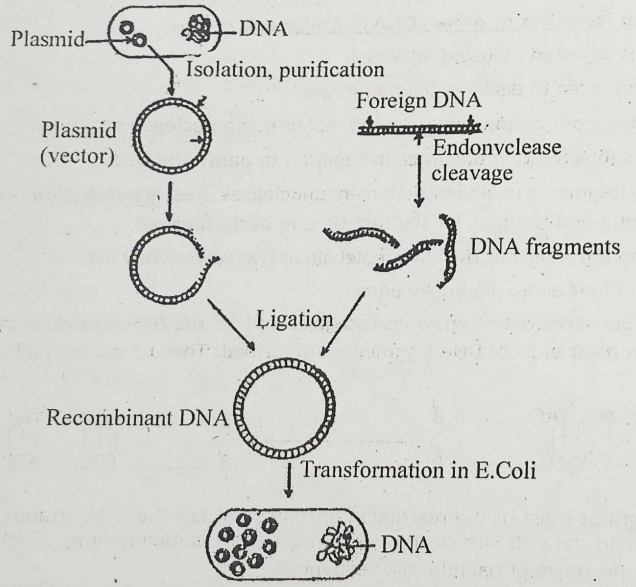
2. Insertion of gene into a suitable vector:

The desired fragment or fragments of DNA are inserted into a suitable vector to produce indefinite number of copies of genes. This is known as gene cloning.

These days gene cloning is performed through polymerase chain reaction (PCR) using thermal cycler. Not only natural vectors, restructured artificial vectors are also used for gene cloning. In which P^{BR322}, P^{UC101} vectors are widely used in gene cloning.

To isolate plasmid, bacterium cell firstly treat with Ethylene Di amine Tetra Acetic Acid (EDTA), then treat with lysozyme enzyme to dissolve cell wall. Later bacterium cell in Sodium lauryl Sulphate subjected to centrifugation to isolate plasmid.

Cleave the plasmid DNA and donor DNA with same restriction enzyme so that identical sticky ends formed in both. The sticky ends of plasmid and desired DNA fragments are annealed through complimentary base pairing and the gaps are joined by DNA ligase. The new plasmid molecule, thus obtained is called recombinant DNA (r-DNA).



Main stages in Gene clioning

3. Introduction of recombinant vector into a suitable host:

When r-DNA and host cell are placed in calcium chloride solution, r-DNA entered into host cell by transformation. Generally Escherichia coli cells are used as host cells to introduce r-DNA. The bacterium cells into which the recombinant DNA is inserted are called transformed cells. This r-DNA starts replication in host cell. Clones are formed in each cell.

4. Selection and screening of transformed host cells: The selection of transformed cells usually depends on the nature of gene which is cloned. For example if it is antibiotic resistance, the cells are first incubated in a medium without antibiotic for about one hour, to allow the antibiotic resistant genes to be expressed. Then the cells are placed on medium with an antibiotic for the selection of colonies containing recombinant DNA.

Applications of r-DNA Technology:

1. Disease resistant varities can be produced through r-DNA technology.
2. Drought tolerant plants can be produced by using r-DNA technology.
3. By using this technology plants with high nutritive value can be produced.
4. Genes useful for nitrogen fixation are called nif genes. This technology helps to introduce nif genes into plants.
5. Human insulin is produced by transformed E. coli bacterium cells having r-DNA that contain insulin gene.
6. Somatotropin hormone which promotes growth in humans produced from E. coli cells.
7. Mice insulin hormone is also produced from E. coli cells.
8. The protein produced by transformed pseudomonas phorascens inhibits the insect growth.
9. A new strain of Colon bacillus is prepared which produce alpha-interferons that inhibit the growth of viruses.
10. Microorganisms which release methane rapidly in biogas plants have been grown.
11. Different types of vaccines are produced by r-DNA technology.

RESTRICTION ENDONUCLEASES

Restriction enzymes are DNA cutting enzymes found in bacteria. Restriction enzymes are first identified by Arber in E. coli bacteria. Because they cut within the molecule, they are often called restriction endonucleases. They are also called as chemical knives. Restriction enzymes are 4 types. They are

1. Restriction endonucleases-I
2. Restriction endonucleases -II
3. Restriction endonucleases-III
4. Restriction endonucleases-IV

1. R.E-I: This type of enzymes has three subunits for three different functions such as recognition of specific sites, cleavage and methylation. These enzymes can cleave up to 1000bp from the recognition site.

2. R.E-II: These enzymes cleave the DNA within their recognition sequences.

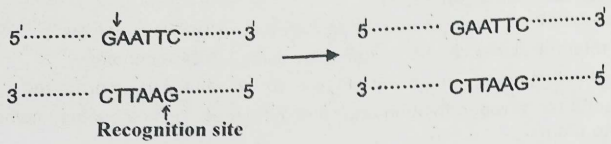
3. R.E-III: These enzymes have two subunits.In which first subunit identifies the recognition sequences and second subunit recognizes cleavage site. Cleavage site is about 25bp distance from recognition site.

4. R.E -IV: In this type, cleavage enzyme is different from methylation enzyme. Recognition site is about

20bp distance from cleavage site.

Recognition Sites:

Restriction enzymes can recognize and cleave at specific sites containing 4 to 6 nitrogen base pairs in DNA. These nucleotide sequences are called recognition site (recognition sequence). The two strands in DNA show same sequence of-nitrogen bases in opposite direction at specific sites. These nitrogen sequences are called palindromic sequences.



Mode of action of Restriction Enzymes:

A restriction enzyme recognizes and cleaves only at particular sequence in DNA. At these specific sites nitrogen base pairs are in same sequence but in opposite directions on two strands. For example, if the sequence is GAATTC on one strand,- the sequence on the other strand is CTTAAG. Restriction enzymes break down the bonds in two strands simultaneously and release the fragments. If the cleavage site is between GA in one strand and in other strands it is AG..

EcoRI recognizes GAATTC sequence and remove the bond between GA. Hind-III enzyme recognizes AAGCTT sequence and remove the bond between GC. Cleaved DNA fragments may have even (Hind-III) or uneven ends (EcoRI). Uneven ends are called Sticky ends.

Enzyme	Recognition site	Products
EcoR - I	5'.....G↓A-A-T-T-C.....3' 3'.....C-T-T-A-A↑G.....5'	5'.....G A-A-T-T-C.....3' 3'.....C-T-T-A-A G.....5'
Hac - III	5'..... G-G↓C-C .....3' 3'..... C-C↑G-G .....5'	5'.....G-G C-C.....3' 3'.....C-C G-G.....5'

Restriction Maps:

DNA molecule can be cleaved at specific sites With the help of restriction enzymes. One can construct gene maps with DNA fragments formed by restriction enzyme digestion. In this way, based on the specific characters of restriction enzymes, gene sites can be recognize on chromosomes and gene maps can be constructed. These maps are called restriction maps. Different sites have about 300bp or less number in DNA fragments can be identified in restriction maps. Later by include these sites, nucleotide sequence of one gene can be decided.

DNA LIGASE (LIGASES)

Enzymes essential to anneal the DNA fragments cleaved by the restriction enzymes are called DNA ligases. These enzymes were firstly isolated from virus (T_4 bacteriophage). These are also found in bacteria (E.coil) and eukaryotic cells. These enzymes repair the DNA molecule. Sticky ends of DNA fragments have complimentary nitrogen bases. Phospo di ester bonds are formed between these complimentary bases. Thus DNA fragments are annealed one with other. DNA ligases anneal DNA fragments without any gaps. DNA ligases are also called as **"chemical sewing machines".**

DNA Ligase : It is the main enzyme in Gene Cloning. It catalyzes the formation of phosphodiester bond between 3'–OH and 5'–PO4 groups of nucleotides and facilitates the joining of DNA fragments. It is also known as a 'Molecular Stitcher'. This ability enables the filling the gaps or the nicks of DNA and joining OKAZAKI fragments formed during DNA replication.

DNA ligases are mainly used in rDNA technology for the production of recombinant DNA molecules during Gene Cloning.

DNA ligase joins DNA of interest (DI or GI) with vector DNA (Plasmids etc.)

DNA ligase depends on a cofactor and Mg_2^+ ions for its function. NAD^+ acts as a cofactor for Bacterial DNA Ligases. While ATP acts as a cofactor for VIRUS and Eukaryotic DNA ligases.

Uses of ligase enzyme:

1. DNA ligases are used to prepare r-DNA by annealing the vector DNA with desired gene.
2. Ligases are used to anneal the two genes from different sources and embedded the desired genes.
3. Blunt ended fragments are changed to sticky ends by annealing the adopters or linkers using DNA ligases.

POLYMERASES

A polymerase is an enzyme that synthesizes long chains of polymers or nucleic acids. DNA and RNA polymerase are the two common types.

DNA Polymerase : It is present in all living organisms. It helps in DNA synthesis and Genome replication. This ability of DNA polymerase is employed in Gene cloning.

This enzyme catalyzes the synthesis of new DNA, complementary for the existing DNA.

DNA Polymerase I of E. Coli directs the synthesis of complementary nucleic acids using single stranded DNA as template. It converts single stranded DNA to the double stranded form. DNA polymerase synthesizes, DNA molecules from deoxyribonucleotides. DNA Polymerase adds nucleotides to the 3'OH (3' end) group of the primer sequence (Bases) and continues the strand formation of 5' direction i.e., elongation of the newly forming strand in a 5'– 3' direction.

Conclusion : DNA ligase helps the joining DNA fragments by phosphodiester bonds, where as DNA polymerase helps the synthesis of new DNA. Though both enzymes are functionally different but they are required for DNA repairing, DNA replication and recombinant DNA technology activities. And these activities are essential for all Gene Cloning procedures in one way or other.

In PCR mediated Gene Cloning technique, a special Taq DNa polymerase is employed.

CLONING VECTORS CHARATERS AND TYPES

Vectors used to produce the copies of desired genes by inserting into the host cell are called cloning vectors. These are also called DNA vehicles. Vectors carry the desired genes along with own genetic material. Plasmids, bacteriophage, cosmids, Ti-plasmids and artificial chromosomes are used as cloning vectors.

Characteristic features of Vectors:

1. The vector should be small.
2. Vector should have one or more unique restriction sites for restriction enzyme recognition.
3. When foreign DNA fragment is inserted, there should be no change in its replication.
4. It is easy to isolate from the host céll.
4. They should have selectable marker genes resistant to antibiotics (ampicillin and tetracycline)
5. Most importantly vectors should have ori genes for replication.

6. Vectors should have tra- genes which can transport into host cells.

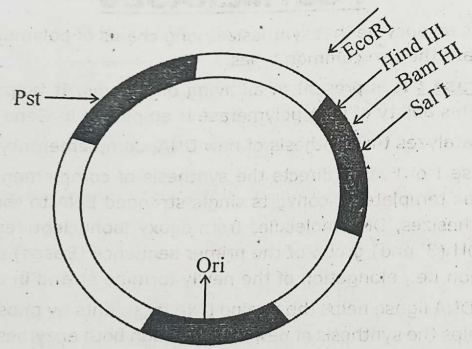
Types of Vectors :

I) Plasmids: plasmids are extra chromosomal, self replicating, circular naked DNA molecules found in the cytoplasm of bacteria. They are about one kbp to 500kbp in length. Plasmids are of two types. They are

i) Conjugated plasmids. ii) Non conjugated plasmids.

As conjugated plasmids have transfer genes they transfer from one bacterium cell to another bacterium cell during conjugation. Non conjugated plasmids do not have transfer genes. Therefore they cannot transfer from one cell to the other. In some bacteria the plasmid number is stable (1 or 2). These plasmids are called stringent plasmids. In some bacteria the plasmid number is more (10- 100). These are known as relaxed plasmids.

In plasmids pBR322 is best example. This plasmid was first constructed by Bolivar (B) and Rodriguez (R) . The plasmid has a point of origin of replication (ori), two selectable marker genes conferring resistance to antibiotics, e.g., ampicillin (amp), tetracycline (tet) and unique recognition sites for 20 restriction endonucleases (EcoRI, HindIII, BamHI, Pst 1 etc.).



PBR^{322}-Plasmid

2. Bacteriophages: Bacteriophages are the Viruses that infect bacteria. These phages are being employed in gene cloning. 50% of the phage DNA is essential for its. replication and other functions. Remaining 50% DNA can be replaced by desired genes.

M_{13} bacteriophage has single stranded DNA which infects E. coli cells . M_{13} bacteriophage synthesizes complimentary DNA strand in host cell. This is called as replicated form of DNA. This DNA is isolated and coverted into recombinant DNA by inserting the foreign DNA fragment. It is introduced into the host cell as hybrid plasmid.

3. Cosmids: cosmids are plasmids that contain phage cos sites and can be packed into phage capsids. They are used to clone large segments of DNA.

Cosmids are plasmids that contain lambda phage DNA containing cohesive ends (Cosmid = cos site + plasmid). For the first time it was developed by Collins and Hohn (1978).

Cosmids lack encoding viral proteins. Therefore, neither viral particles are formed nor cell lysis occurs. Cosmids like plasmids contain –

(i) Origin of replication,

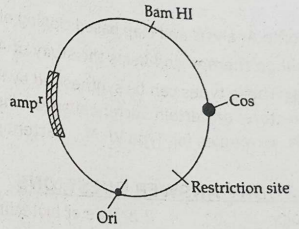
(ii) a marker gene coding for antibiotic resistance,

(iii) a special cleavage site for the insertion of foreign DNA,

(iv) The small size.

They differ from plasmids in having are extra phase DNA, the cos site, which is about 12 bases. It helps the whole genome in circularization and ligation.

e.g. Col E 1 cosmid ⟶ ColE 1+ λ phage DNA



Structure of Col E 1 cosmid

4. Phagemids: These are artificially prepared vectors like cosmids, by combing the features of phages with plasmids.

Characters of Phagemids : These possess –

(a) A multiple cloning site.

(b) An inducible promoter

(c) An origin of replication (Derived both from phage and plasmid)

Example for this type is p Blue script 11 ks : It is derived from the plasmid pUC19 type and is 2961 bp long. The KS designates the orientation of polylinker.

5. Larger DNA fragments cloning vectors: These vectors are very useful for characterization and expression of large genes or gene complexes. A number of such vectors have been developed recently to utilize on large geonomes of Humans and Mammalia.

Some of the popular examples for this type are YACs, PACs, BACs etc.

YAC (Yeast Artificial Chromosome) : YAC can clone very large DNA fragments upto 2 Mb. This character of YAC has made it a vital tool in creating physical maps of large genomes such as the human genome. Each YAC is made up of 3 regions, namely Telomere (TEL), Centromere (CEN) and a replicating sequence (RS). Hence YAC is described as a mini chromosome. It behaves as an additional chromosome.

Uses of YAC :

1. It is used to form Genomic libraries of both Pro and Eukaryotes.
2. It is used in Human Genome Project (HGP) to construct Gene Map of Chromosome in Man.
3. It is used to clone large DNA fragments.

PAC: It is called P_1– DErived Artificial Chromosome. PACs are DNA constructs that are derived from the DNA of P_1–bacteriophage. They can carry large amounts (100–300Kbs) of other sequences for variety of Bio engineering purposes. It is one type of vector to clone DNa fragments in E. Coli cells.

This vector (PAC) originates from a phage (P_1 Type) instead of a plasmid. P_1Phage is unique in nature and exists in two forms. In Lysogeny, it remains and acts like a plasmid and in Lysis, it destroys the Host Cell.

PAC contains both features of Plasmids and F factor (F=fertile plasmid like DNA sequence) i.e., P and F factor systems.

PAC can accommodate larger inserts of DNA than a plasmid or many other vectors.

PACs are constructed through a method called 'Electroporation'.

Uses or Applications :

1. PACs are used in the Genome Analysis and Map based cloning of Complex Plants and Animals.
2. PAC cloning is useful in phage therapy and helps the study of Antibiotic actions.

6. ssDNA preparing Vectors: These types can be synthesized by PCR–based methods. Besides, these can be obtained, using vectors, on certain bacteriophages, whose genomes assume a single stranded DNA in their life cycle. Examples for Type VI–M_{13} Bacteriophages, M_{13} Vectors.

SHORT ANSWER QUESTIONS

1. Applications of biotechnology
2. Role of biotechnology in agriculture
3. Role of biotechnology in medicine
4. Role of biotechnology in industry
5. Applications of.r-DNA.
6. Restriction endonucleases
7. Ligages.
8. Polymerases
9. r-DNA technology (gene cloning)
10. Plasmid
11. Bacteriophage.
12. Cosmid
13. Larger DNA fragments cloning vectors

LONG ANSWER QUESTIONS

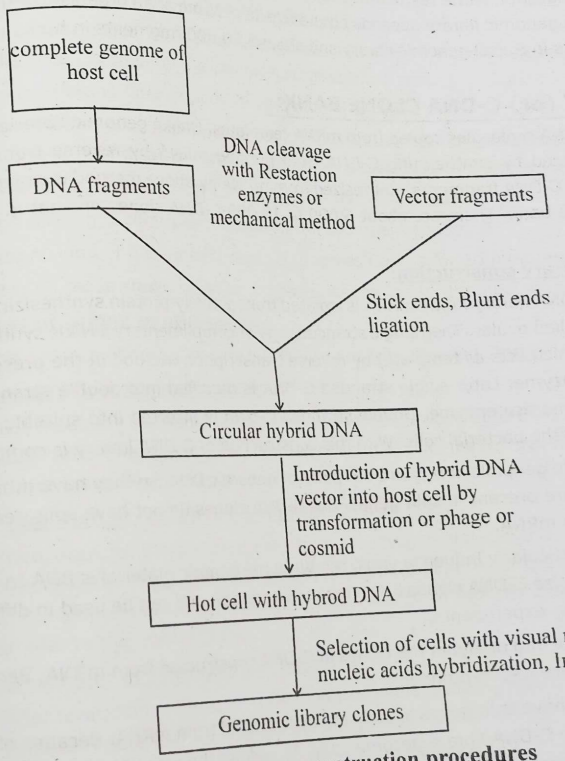
1. What is biotechnology? Explain the role of biotechnology in different fields.
2. What is r-DNA technology? Mention different stages and applications of r-DNA technology.
3. Write about restriction endonucleases , Ligages, Polymerases enzymes in r-DNA technology.
4. Define cloning vectors? Write about various cloning vectors.

UNIT-IV

GENOMIC LIBRARIES-DNA LIBRARIES

Collection of the DNA clone fragments of all genes of the genome of an organism is called gene bank or genomic library. The genomic library can be prepared by the complete digestion of the total genomic DNA with a restriction enzyme. The fragments of desired size are collected and can be cloned directly.

Construction of genomic library:



Genomic library construction procedures

Usually Shotgun experiment is an important method in construction of genomic library. Shotgun experiment is the method of collective cloning of complete genes (without recognize) in a genome of an organism. Construction of genomic library involves following steps.

1. Isolation of target genomic DNA.
2. Cut the DNA into fragments of different length using restriction enzymes.
3. DNA fragments are inserted into suitable vectors for cloning to chimeric DNA.
4. Vector with chimeric DNA is introduced into suitable host cell for cloning.

5. Screening of desired clones (having desired genes).

6. Genomic library constructed with selected clones of an organism can be seen in following figure.

.Different types of restriction enzymes are used simultaneously in genomic library construction, as a result gene can be cleaved irregularly at different sites and form more fragments. It may not possible to add all these fragments at one place. However, by making some following changes in shotgun method genomic library can be constructed.

i) Use of restriction enzymes which recognize short length (4bp) sequences.

ii) Genomic library can construct by taking proper care while restriction digestion. Due to partial restriction digestion same restriction site will not to cleave several times. The number of DNA fragments in a genomic library depends on the size-of genome of an organism. For example 1500 DNA fragments in E. coli genomic library and about 8,00,000 fragments in human genomic library.

C- DNA LIBRAY (or) C-DNA CLONE BANK:-

C-DNAs are DNA molecules copied from mRNA templates. Unlike genomic libraries C-DNA libraries are constructed by synthesizing C-DNA from purified mRNA by reverse transcription method. The group of C-DNA fragments synthesized from mRNA by above method is called C-DNA library or C-DNA clone bank. There are about 5,000 to 10,000 C-DNA clones present in each C-DNA library.

Process of C-DNA library construction:

To construct C-DNA library firstly mRNA is isolated from actively protein synthesizing plant roots, leaves or mammalian ovules. Then single stranded copy or complimentary DNA is synthesized from isolated mRNA (which acts as template) by reverse transcription method in the presence of reverse transcriptase enzyme. Later single stranded C-DNA is modified into double stranded C-DNA by using DNA polymerase enzyme. Double stranded C-DNA is inserted into suitable vector and cloned this C-DNA in the bacterial cells. With this construction of C-DNA library is completed.

As the clones in the genomic library are the parts of genomic DNA, so they have introns or non coding regions that are present in split genes. But C-DNA clones do not have split genes as they are synthesized from mRNA.

In some viruses particularly influenza virus, reo Virus the genetic material is RNA.In these viruses it is easy to synthesize C-DNA clones by reverse transcription and can be used in different types of genetic engineering experiments.

It is easy to recognize and analyzes the clones in C-DNA constructed from mRNA. Because in which C-DNA fragments are less in number.

C-DNA clones do not have split genes as they are synthesized from mRNA. Because of this screening of desired genes in C-DNA library become easy. As well as the lack of introns in the C-DNA, it is suitable to use as probe.

COLONY HYBRIDISATION

- Colony hybridizations is the method first given by the scientists Grinstein and Hogness in the year 1975.
- This method isolates the specific DNA sequences or genes from the hybrid DNA.
- Colony hybridization involves replicas preparation on the nitrocellulose membrane filter paper.

Definition of colony Hybridization :

- Colony hybridization is the blot analysis technique where the bacterial cells are transferred from the solid nutrient medium to the absorbent material.
- It can define the method for isolating the specific DNA sequences or genes from the bacterial cells containing hybrid DNA using a nitro cellulose membrane filter (Transferring medium).

Transferring medium :

- The nitro cellulose filter paper is the transferring medium of the colony hybridization that forms the master plate's replicas.
- Nitro cellulose Filter paper acts as the blotting – pad.

Properties of the nitrocellulose Filter paper :

- The nitrocellulose filter paper comprises 100% pure nitrocellulose cohere the cellulose undergoes nitrogen by the chemical reagent nitric acid.
- This membrane filter provides a high quality transfer.
- Nitrocellulose filter paper possesses a 0.45 µm pore size that facilitates efficient transferring for colony hybridization Nitrocellulose filter paper goes through several steps.
- Three times washing of the filter paper with distilled water.
- Next placing of filter paper between the absorbent sheets.
- Auto claving of filter medium at 120 degrees Celsius for 10 minutes.
- After all these steps, transfer the bacterial cells on to the filter membrane.

Process of colony Hybridization :

- The process of colony hybridization involves the following steps.

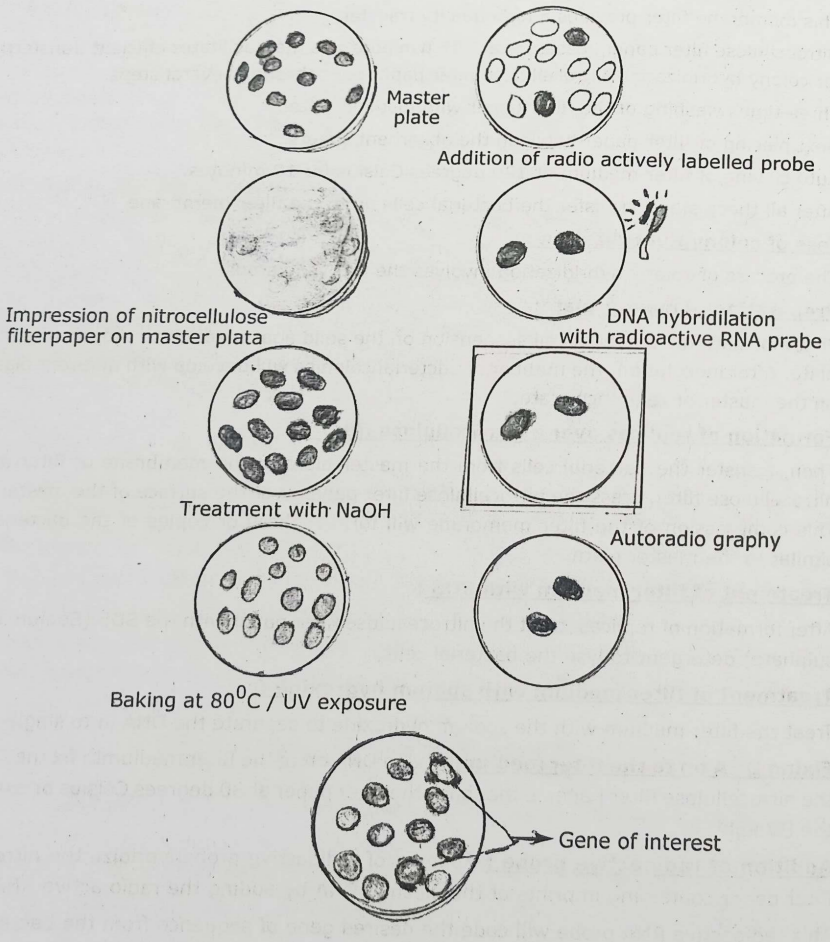
1. **Preparation of master plate:**
   - First inoculate the bacterial cell suspension on the solid agar medium to prepare the master plate. After inoculation, the number of bacterial colonies will develop with different plasmids on the master or reference plate.
2. **Formation of replicas over a nitrocellulose filter :**
   - Then, transfer the bacterial cells from the master plate to the membrane or filter using a nitrocellulose filter, press the nitrocellulose filter paper over the surface of the master plate. This compression of the filter membrane will form replicas or copies of the bacterial cells similar to the master plate.
3. **Treatment of filter medium with SDS :**
   - After formation of replicas, treat the nitrocellulose filter paper with the SDS (Sodium dodecyl sulphate) detergent to lyse the bacterial cells.
4. **Treatment of filter medium with sodium hydroxide :**
   - Treat the filter medium with the sodium hydroxide to separate the DNA in to single strands.
5. **Fixing DNA on to the filter medium:** Fixing DNA on to the filter mediumto fix the DNA onto the nitrocellulose filter paper, either back the filter paper at 80 degrees Celsius or expose it to the UV light.
6. **Addition of radioactive probe :** Addition of radioactive probehybridize the nitrocellulose filter paper containing imprints of the plasmid DNA by adding the radio active RNA probe.
   - This radioactive RNA probe will code the desired gene of sequence from the bacterial cells.

7. Washing and autoradiography :

- Wash the filter paper to remove unbound probe particles, After that, expose the nitro cellulose filter paper to the X-ray film by the method called autoradiography. The colonies that appear after autoradiography are known as auto radio gram.

8. Identification of the desired gene :

- Compare the developed auto radiogram with the master plate to identify the colonies containing a gene of interest. The cells containing the desired genes will grow in the liquid medium, and they can be further processed for the isolation of recombinant plasmid DNA. This colony hybridization method is the screening technique that uses the radioactive probe.
- The radio actively labelled probe screens or isolates the particular gene from the number of bacterial colonies.



OLIGONUCLEOTIDE – PROBE

- Probe is a single stranded polynucleotide of DNA or RNA probes can be composed of CDNA (produced from mRNA by reverse transcriptase).
- Fragments of genomic DNA (Cleaved by restriction enzymes from the genome)
- In molecular biology a probe is a fragment of DNA or RNA of variable length.
- Variable length of probe is usually 100-1000 bases long, it can be radioactively labelled.

Types of Probes :

1. Oligonucleotide probe
2. DNA probes
3. RNA probes

- **Oligonucleotide probes :** A short sequence of nucleotides that are synthesized to match a specific region of DNA (or) RNA then used as a molecular probe to detect the specific DNA or RNA sequence.
- Oligonucleotide probes are generally targeted to specific sequences within genes. The most common oligonucleotide probes contain 18-30 bases, but current synthesizers allow efficient synthesis of probes containing at least 100 bases.
- The probe length should be between 18 and 50 bases. Longer probes will result in longer hybridization times and low synthesis yields, shorter probes will lack specificity.
- The base composition should be 40-60% G-C. Nonspecific hybridization may increase for GC ratios outside of this range.
- Be certain that no complementary regions within the probe are present. These may result in the formation of 'hairpin' structures that will inhibit hybridization to target.
- Avoid sequences containing long stretches (more than four) of a single base once a sequence meeting the above criteria has been identified computerized sequence analysis is highly recommended. The probe sequence should be compared with the sequence region or genome from which it was derived, as well as to the reverse complement of the region. It homologies to non target regions greater than 70% or 80% or more bases in a row are found, that probe sequence should not be used.

POLYMERASE CHAIN REACTION (PCR)

Generating lakhs of copies of double stranded DNA within few hours is called **'polymerase chain reaction or gene amplification'**. PCR was developed by Karry Mullis in 1984.

The double-stranded DNA of interest is denatured to separate into two individual strands. These strands act as templates. By utilizing free nucleotides (ATP, GTP, CTP, TTP) these strands form double stranded DNAs. In this way in a short span time two become four. four become eight and so on.

There are three steps in PCR repeated again and again to generate multiple forms of target DNA. They are.

1. Denaturation 2. Renaturation 3. Synthesis

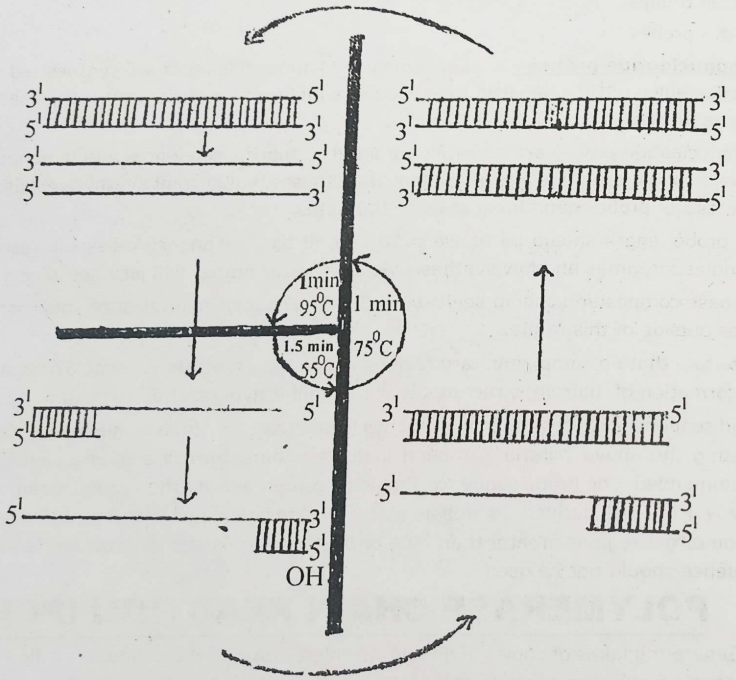
1. Denatu ration: On raising the temperature to about 95°C for about one minute, the DNA gets denatured and the two strands separate. These two strands act as templates.

2. Renaturation: As the temperature of the mixture is slowly cooled down to 55° C for about 1.5 minutes, the primers base pair with the complementary regions flanking target DNA strands. This

process is called renaturation or annealing.

3. Synthesis: The initiation of DNA synthesis occurs at 3-hydroxyl end of each primer. The primers are extended by joining the bases complementary to DNA strands. For this stage the mixture is kept at 75° C for I minute.

PCR cycle is completed in three to five minutes. These new strands are referred to as long templates and they will be used in the second cycle. In second cycle short templates are formed to long templates. The length of the short templates is extended in third cycle.



PCR - Cycle

Advantages of PCR:

1. Gene cloning can be done in small size DNA
2. Variations in DNA can be recognized using either known DNA sequences, cleaved DNA fragments.
3. Nucleotide sequences in a gene can be discovered through this process.
4. More number of copies of DNA can be prepared with minimal technical skill, time and low cost.
5. Aberrations in DNA fragments can be found.
6. Genetic disorders can be detected by this process.

7. Gene expression during embryo development can understand through this process.
8. Nowadays PCR technology is widely used in forensic science.

AGROBACTERIUM-MEDIATED GENE TRANSFER IN HIGHER PLANTS

DNA with desired genes in an organism can be transferred to plants through vectors. Ti plasmid of Agrobacterium tumefaciens is used to transfer the genes in dicots. This is a soil-borne, Gram-negative bacterium. It is rod shaped and motile. Agrobacterium infects wounded or damaged plant tissues, induces the formation of a plant tumor called crown gall. T-DNA fragment of Ti-plasmid is the reason for crown gall disease. In genetic engineering onco genes in T-DNA are removed and replaced by our desired genes. This transformed T-DNA is introduced into. the plant cell. Thus, genetically modified plants with our desired genes are formed.

Ti-Plasmid: Smith and Townsend (1907) postulated that a bacterium was the causative agent of crown gall tumors. As Agrobacterium tumefaciens infects wounded or damaged plant tissues, induces the formation of a plant tumor called crown gall. The entry of the bacterium into the plant tissues is facilitated by the release of certain phenolic compounds by the wounded sites. Crown gall formation occurs when the bacterium releases its Ti plasmid into the plant cell cytoplasm. A fragment of Ti plasmid, referred to as T-DNA, is actually transferred from the bacterium into the host where it gets integrated into the plant cell chromosome. The circular DNA present in the cytoplasm of Agrobacterium tumefaciens is called Ti-plasmid. Ti plasmid consists of four main regions. They are

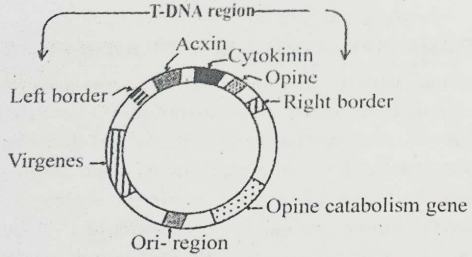
1. T-DNA region
2. Virulence region
3. Opine catabolism region.
4. Ori. region

1. T-DNA region: This region is about 35kbp in size. This region has the genes for the biosynthesis of auxin, cytokinin and opine, and is flanked by left and right borders. These three genes-aux, cyto and ocs are referred to as onco genes, as they are the determinants of the tumour phehotype. It is now clearly established that the right border is more critical for T-DNA transfer and tumour-genesis.

2. Virulence region: The genes responsible for the transfer of T-DNA into the host plant are located outside T-DNA and the region is referred to as vir or virulence region. Vir region codes for proteins involved in T- DNA transfer. At least nine vir-gene operons have been identified. These include vir A, vir G, vir B_1, vir C_1, vir D_1, D_2 and D_4, and vir E_1, and E_2.

3. Opine metabolism region: The genes this region codes for proteins involved in the uptake and metabolisms of opines.

4. Ori region: Besides the above three, there is ori region that is responsible for the origin of DNA replication.



Ti -Plasmid

T-DNA transfer and integration into host genome have following stages:

1. To establish relation with plant by Agrobacterium.
2. Attachment of Agrobacterium to plant cells
3. Production of virulence proteins:
4. Production of T-DNA strand:
5. Transfer of T-DNA out of Agrobacterium:
6. Transfer of T-DNA into plant cells and integration

1. To establish relation with plant by Agrobacterium: The wounded plant cells release certain chemicals- phenolic compounds and sugarswhich are recognized as signals by Agrobacterium. The signals induced result in a sequence of biochemical events in Agrobacterium that ultimately helps in the transfer of T-DNA of T-plasmid.

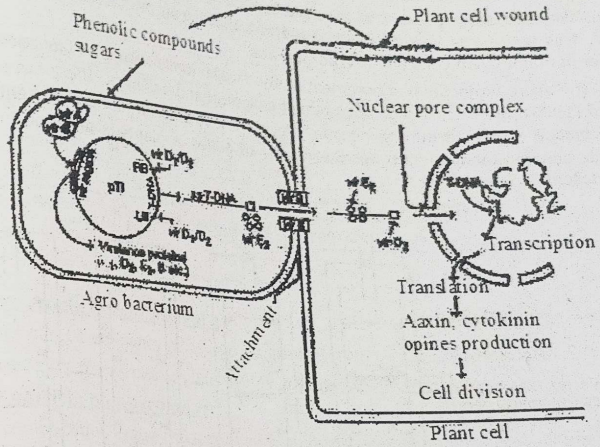
2. Attachment of Agrobacterium to plant cells: The Agrobacterium cell wall contains lipopolysaccharides. These polysaccharides establish a relation with polygalacto uronic acid in the plant cell wall. As a result bacterium attaches to the plant cell wall.

3. Production of virulence proteins: Cell walls of the wounded cells secrete phenolic compound acetosyrinzone, which activates virgenes. As a result virulence proteins like D_1, D_2, E_2, B etc. are synthesized.

4. Production of T-DNA strand: The right and left borders of T-DNA are recognized by vir D_1/vir D_2 proteins. These proteins are involved in the production single-stranded T-DNA (ss DNA), its protection and export to plant cells. The ss T-DNA get attached to vir D_2.

5. Transfer of T-DNA out of Agrobacterium: The ss T-DNA- vir D_2 complex in association with vir G is exported from the bacterial cell. Vir B products form the transport apparatus.

6. Transfer of T-DNA into plant cells and integration: The T-DNA-vir D_2 complex crosses the plant plasma membrane. In the plant cells, T-DNA gets covered with vir E_2. This covering protects the T-DNA from degradation by nucleases; vir D_2 and vir E_2 interact with a variety of plant proteins which influences T-DNA transport and integration. The T- DNA-vir D_2-vir E_2 -plant protein complex enters the nucleus through nuclear pore complex. Within the nucleus, the T-DNA gets integrated into the plant chromosome through a process referred to illegitimate recombination. This is different from the homologous recombination, as it does not depend on the sequence similarity.



PHYSICAL GENE TRANSFER METHODS

In this three main, popular methods are described in detail below. They are :

(1) Electroporation. (2) Microprojectile Bombardment (3) Microinjection.

1. Electroporation : Electroporation basically involves the use of high field strength electrical impulses to reversibly permeabilize the cell membranes for the uptake of DNA. This technique can be used for the delivery of DNA into intact plant cells and protoplasts.

The plant material is incubated in a buffer solution containing the desired foreign / target DNA and subjected to high voltage electrical impulses. This results in the formation of pores in the plasma membrane through which DNA enters and gets integrated into the host cell genome.

In the early years, only protplasts were used for gene transfer by electroporation. Now a days, intact cells, callus cultures and immature embryos can be used with suitable pre- and post-electroporation treatments. Electroporation has been successfully used for the production of transgenic plants of many cereals e.g. rice, wheat, maize.

Advantages of electroporation :

1. This technique is simple, convenient and rapid, besides being cost-effective.
2. The transformed cells are at the same physiological state after electroporation.
3. Efficiency of transformation can be improved by optimizing the electrical field strength and addition of spermidine.

Limitations of electroporation :

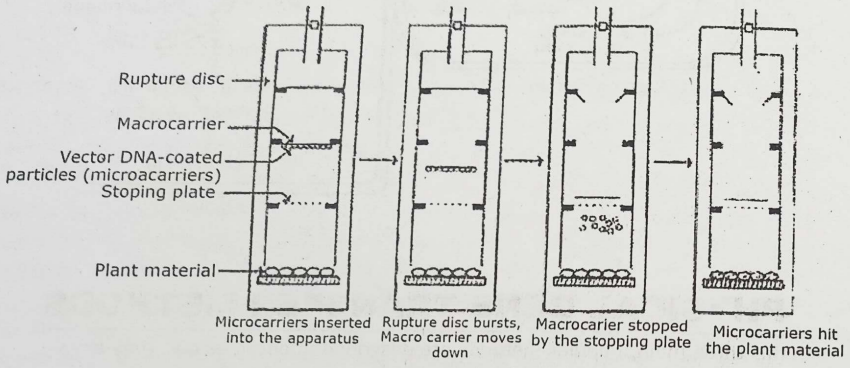
1. Under normal conditions, the amount of DNA delivered into plant cells is very low.
2. Efficiency of electroporation is highly variable depending on the plant material and the treatment conditions.

3. Regeneration of plants is not very easy, particularly when protoplasts are used.

2. Microprojectile Method (Particle Bombardment (Biolistics) :

Micro projectile is the most effective method for gene transfer and creation of transgenic plants. This method is versatile due to the fact that it can be successfully used for the DNA transfer in mammalian cells and microorganisms.

The micro projectile bombardment method was initially named as biolistics by its inventor Sanford (1988). Biolistics is a combination of biological and ballistics. There are other names for this technique - particle gun, gene gun, bio blaster. A diagrammatic representation of micro projectile bombardment system for the transfer of genes in plants is depicted in Figure. A and briefly described below.



A diagrammatic representation of particle bombardment (biolistics) or Microprogectile system for gene transfer in plants.

Micro carriers (micro projectiles), the tungsten or gold particles coated with DNA, are carried by macro carriers (macro projectiles). These macro-carriers are inserted into the apparatus and pushed downward by rupturing the disc.

The stopping plate does not permit the movement of macro carrier while the micro carriers (with DNA) are propelled at a high speed into the plant material. Here the DNA segments are released which enter the plant cells and integrate with the genome.

Plant material used in bombardment :

Two types of plant tissue are commonly used for particle bombardment.

1. Primary explants which can be subjected to bombardment that are subsequently induced to become embryogenic and regenerate.
2. Proliferating embryonic tissues that can be bombarded in cultures and then allowed to proliferate and regenerate.

In order to protect plant tissues from being damaged by bombardment, cultures are maintained on high osmoticum media or subjected to limited plasmolysis.

The success of bombardment : The particle bombardment technique was first introduced in 1987. It has been successfully used for the transformation of many cereals, e.g. rice, wheat, maize. In fact, the first commercial genetically modified (GM) crops such as maize containing Bt-toxin gene were developed by this approach.

A selected list of the transgenic plants (developed by biolistics) along with the sources of the plant materials used is given in Table A.

Table 'A' selected list of transgenic plants (along with cell sources) developed by microprojectile bombardment)

	Plant	Cell source (s)
1	Rice	Embryonic callus, immature zygotic embryos
2	Wheat	Immature zygotic embryos
3	Sorghum	Immature zygotic embryos
4	Corn	Embryonic cell suspension, immature zygotic embryos
5	Barley	Cell suspension, immature zygotic embryos

Advantages of particle bombardment :

1. Gene transfer can be efficiency done in organized tissues.
2. Different species of plants can be used to develop transgenic plants.

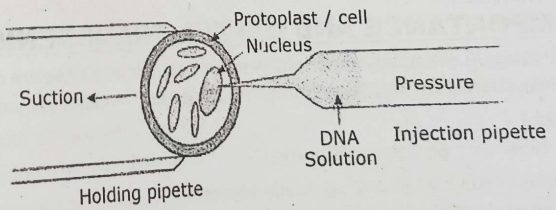
Limitations of particle bombardment :

1. The major complication is the production of high transgene copy number. This may result in instability of transgene expression due to gene silencing.
2. The target tissue may often get damaged due to lack of control of bombardment velocity.
3. Sometimes, undesirable chimeric plants may be regenerated.

3. Microinjection :

Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell. The target cell may be the one identified from intact cells, protoplasts, callus, embryos, meristems etc. Microinjection is used for the transfer of cellular organelles and for the manipulation of chromosomes.

The technique of microinjection involves the transfer of the gene through a micropipette (0.5–10.0 pm tip) into the cytoplasm / nucleus of a plant cell or protoplast. While the gene transfer is done, the recipient cells are kept immobilized in agarose embedding and held by a suction holding pipette.



Microinjection of DNA by holding pipette method

As the process of microinjection is complete, the transformed cell is cultured and grown to develop into a transgenic plant. In fact, transgenic tobacco and Brassica napus have been developed by this approach. The major limitations of microinjection are that it is slow, expensive and has to be performed by trained and skilled personnel.

GENETICALLY MODIFIED PLANTS (OR) TRANSGENIC PLANTS

Transgenic plants are plants into which one or more desired genes from other species have been introduced into the genome using genetic engineering processes. This process is called plant transgenesis. The development of transgenic plant utilises the genetic engineering technology through tissue culture methods. These plants are used to create agriculture, horticulture, ornamentally valuble plants and for the production proteins, medicines, drugs. Similarly these plants are created to know about gene action in plant development. Nowadays many types of genetically modified plants are created. They are respectively disease resistant, fungal resistant, herbicide resistant, plants with high nutritive value, abiotic stress resistant plants, plants showing self incompatibility.

Creation of transgenic plants

Transgenic plants are created by introducing different types of desired genes into plants through direct or indirect gene transfer methods. Agrobacterium, cauliflower mosaic virus, Gemini virus and RNA viruses are used as vectors to prepare transgenic plants. Similarly direct gene transfer methods like electrophoresis, microinjection, liposome, PEG, calcium phosphate are used to transfer desired genes. Genetically modified plants are created by using marker genes like antibiotic resistant (neomycin, hygromycin) and reporter genes which form color or light luminous genes are used to select transformed cells from non transformed cells, promoter and terminator genes.

Applications of transgenic plants:

1. Transformed plants with Bt-gene show insect resistance. Genetically (bollguard) modified Bt-cotton plant has resistant against bollworm insect. Bt-toxin is quickly degraded in the nature.
2. Virus coat protein genes have been introduced into crop plants like rice, wheat, potato, sugar beet. These genetically modified plants have resistance to virus.
3. Genes which inactivates chitinase, glucanase, lysozyme and ribosome are transferred to tobacco, paddy, carrot, tomato, potato plants. These genetically modified plants exhibit resistance to bacterial and fungal diseases.
4. High yielding and quality transgenic plants created in tomato and potato. In transgenic tomato (Flavr Savr) ripening is delayed. So that shelf life is increased and can export to far away areas.
5. Transgenic plants are used as bioreactors. These plants are useful in production of important chemicals. The important chemicals produced by these plants are sugars, lipids, proteins, antibodies, vaccines etc.

SPECIAL IMPORTANCE AND USEFUL TRANSGENIC PLANTS

- These, with distinctive examples, are briefly described as follows. These are divided into four groups and their characteristics, temperaments, actions and benefits are explained.

Community types :

1. Pest resistant type. Ex : Bt-Cotton, Bt-Brinjal
2. Herbicide resistant type Ex : Round up Ready soyabean
3. Enhanced (Develop made) agronomic type. Ex : Flavr, savr, Tomato, Golden rice.
4. Enhanced horticulture types

1. Pest resistant type : Ex : Bt-Cotton, Bt-Brinjal

Bt – Gene: Genes establishing insect resistance in Tansgenic plants has been achieved through the use of insect control protein genes. These genes have been obtained from micro-organisms.

Examples : (1) Bt gene from Bacillus thuringiensis.

(2) iBt gene from Agrobacterium tumefaciens.

Bt– gene - Mode of Activity : B. Thuringiensis (Bt) is an entomocidal bacterium that produces an insect control protein. Bt genes code for the 'Bt TOXIN'. Most of the Bt TOXINS are active against Lepidopteran larvae but some are specific for Dipteran and Colopteran insects. Bt Toxin accumulate as crystal proteins. These are designated as Cry 1, Cry 2, Cry3 etc. types. These ultimately kill the infects insects by causing disruption in their cell membranes etc.

Bt–Cotton : It is genetically modified (Transgenic) pest resistant cotton variety, which produces insecticide to Bollworm.

The gene coding for Bt Toxin has been inserted into cotton, as a Transgene, causing it to produce insecticide in its tissues. Bt cotton protein kills larvae of Lepidopteran.

Bt cotton is created by the addition of Bt genes encoding toxin crystals in the Cry group of endotoxin. Cry 1 Ac is a specific toxin of Bt–Cotton, which is highly toxic to Cotton Bollworm and kills the infecting pathogen as explained before in Bt–gene–Mode of Activity.

Applications of Bt–Cotton : Bt–cotton has several advantages over non–Bt–cotton plants. The important applications / advantages of Bt–cotton are as follows :

1. The effective control of Boll-worms by Bt–cotton leads to the increase in yield of cotton that is under cultivation.
2. It reduces the cost of cultivation of cotton crops.
3. Cultivation of Bt–cotton varieties indirectly reduces the use of insecticides.
4. Bt. Cotton varieties exhibit high resistance to different environmental factors compared to non-Bt-cotton varieties.

Bt–Brinjal :

- Shigetane I shiwatari (1901). First isolated bacillus thuringinsis.
- Bt is commonly abbreviated as bacillus thuringiensis, is gram-positive, facultative aerobic, rod-like, motile and sporulating bacterium.
- Bt is a naturally occurring soil borne bacterium that is found world wide.
- Ubiquitous in nature.
- Produces crystals of endotoxin (cry protein or delta toxin) – toxic to insect mainly in their larval stage, thus they act as insecticides.
- These crystal proteins (cry proteins) are insect stomach poisons.
- So the insects stop feeding within two hours of a first bite and, if enough, toxin is eaten, die within two or three days.
- Important biological insect control agent.
- Bt. Crystals, some times referred as insecticidal crystal proteins (I cp), are protein crystals formed during sporulation in some Bt. strains coded by cry genes.
- Coming to the BT. Brinjal.
- Bt. Brinjal is a transgenic brinjal.
- Brinjal is botanically known as solanum melongena l (2n=24). Induced under the solanum. Which is of the largest genera having more than 1500 described plant species.
- The cultivated types are fit for human consumption and are divides to three main varieties based on fruit shape. They are.
- 1. S. melongena var esculentum shows round (or) egg. Shape.
- 2. S. Melongena var serpentinum shows long and slender shape.
- 3. S. Melongena var depressor shows dwarf brinjal plants.

Benefits of Bt. Brinjal :

- Bt. brinjal plants resistance against lepidopteran insects.
- Non-infested, undamaged and good quality brinjal fruits an produced.
- Bt. Brinjal will significantly reduced level of pesticide residues, formers and expected to benefit at multiple levels. Some of these include.
- Saving on cost of insecticides and lower labour cost as a result of reduced spraying. Increase in yield per unit area by saving fruits from damage by fruit shoot b over (FSB).
- Bt. has no toxic impact in higher animals (Human) because of Acidic stomach and very low pH (1.5) & absence of required receptors.

2. Type of herbicide resistant: Ex: Round Up Ready soybean(RR Types)

RR soybeans are identified as variants of DNA modified by a genetic engineering process.Soybeans are resistant to glyphosate, so the varieties are called glyphosate tolerant soy beans.

When glyphosate is sprayed on fields where RR soayabeans grow, only the weeds in the field are killed. Only RR soayabeans grow normally.High yields due to cultivation of RR varieties have been identified at the field level.

3. Enhanced Agronomic type Ex : Golden rice :

Ex : Flavr savr Tomoto

a. Flavr Savr Tomato : (Flavor, savor)

- This is called (GM) a Genetically modified tomato variety. During this type of development / growth, it exhibits a slow ripening nature / characteristic.
- It softens the plaque and prevents process / action. But the colour and aroma of this tomato remain the same.
- This GM tomato variety has been shown to be resistant to Rotting action.
- For that, a unique anti sense gene was added to this GM. This gene inhibits the production of an enzyme called PGU (Poly galacturonate) usually PGU (Poly galacto uronase) degrades the pectin substance present in the cell membranes. The result is that the membranes soften. But the tenderness (soften) of the GM tomato is not caused by the Antisensengene.

(b) Golden Rice :

Transgenic golden rice, produced by the GE process, is produced by the biosynthesis of beta carotene, only in the edible components of rice. Here Beta carotene is a precursor to Vitamin-A.

- Golden rice has been recognised as the result of an experimental effort to produce transgenic rice varieties to correct vitamin a deficiencies in the diet.
- The golden rice variety contains chemically structured 3-beta carotene biosynthetic genes. The Golden Rice variety was hybridized with maize to grow a transgenic variety called Golden Rice-2. It contains more carotene than the first type of golden rice.

4. Enhanced Horticulture types :

The science of growing fruits, vegetables, and ornamental plants today is defined as horticulture.

Many genetically modified horticultural plants have been developed in to transgenic varieties. These are called GM horticultural varieties. They are divided into a few complexes.

Respectively they are (1) Herbicide inhibitors (2) Pesticide (Insecticides) inhibitors (3) Disease inhibitors (4) Types of tolerance of inanimate objects. (5) Types of floral color metamorphosis (6) Types of Fragrance transformation (7) These are production pathways of edible vaccines.

SHORT ANSWER QUESTIONS

1. DNA libraries.
2. Colony hybridisation
3. Oligonucleotide -probe
4. PCR
5. PCR Application
6. Electroporation
7. Mibroprojectile
8. Microinjection
9. Transgenic plants
10. Transgenic plants applications
11. Bt Cotton
12. Bt Brinjal
13. Falvr savr tomato
14. Golden rice

LONG ANSWER QUESTIONS

1. What are genomic libraries? Write about genomic library, C-DNA library.
2. Explain Colony hybridisation.
3. Define PCR? Write about its stages and uses.
4. Write about the gene transformation process through Agrobacterium in higher plants.
5. Explain Physical gene transfer methods.
6. What are transgenic plants? Mention about its production and applications.
7. Explain special importance and useful transgenic plants.

8. a) Define and explain triploids.

(or)

b) What is cytoplasmic hybridization (or) Cybrids? Mention its procedure, applications.

9. a) What is biotechnology? Explain the role of biotechnology in different fields.

(or)

b) What is r-DNA technology? Mention different stages and applications of r-DNA technology.

10. a) Write about the gene transformation process through Agrobacterium in higher plants.

(or)

b) Explain special importance and useful transgenic plants.

Osmania University, Palamuru University, Telangana University, Shatavahana University, Kakatiya University

Time: 3 Hrs — VI SEMESTER MODEL PAPER -I — Max. Marks: 80

PART-'A' SHORT QUESTIONS (8X4=32 Marks)

I. Answer any eight from the given questions.

1. a) Micro propagation
   b) Leaf culture
   c) Organogenesis
   d) Production of pathogen free plants
   e) Production of secondary metabolities by hairy root culture
   f) Cybrids
   g) Applications of biotechnology
   h) Applications of.r-DNA.
   i) Polymerases
   j) DNA libraries.
   k) PCR Application
   l) Bt Cotton

PART-'B' LONG QUESTIONS (4X12=48 Marks)

II. Answer all the questions.

2. a) What is tissue culture? Write about the different stages in tissue culture.

(or)

b) Define and explain Organogenesis?

3. a) What is tissue culture? Mention its application.

(or)

b) What is somatic hybridization? Write about its procedure, applications.

4. a) What is biotechnology? Explain the role of biotechnology in different fields.

(or)

b) Define cloning vectors? Write about various cloning vectors.

5. a) What are genomic libraries? Write about genomic library, C-DNA library.

(or)

b) What are transgenic plants? Mention about its production and applications.

Osmania University, Palamuru University, Telangana University, Shatavahana University, Kakatiya University

Time: 3 Hrs — VI SEMESTER MODEL PAPER -II — Max. Marks: 80

PART-'A' SHORT QUESTIONS (8X4=32 Marks)

I. Answer any eight from the given questions.

1. a) Preparation of culture medium
   b) Root culture
   c) Callus culture
   d) Synthetic seeds
   e) Haploids
   f) Somatic hybridization
   g) Role of biotechnology in agriculture
   h) Restriction endonucleases
   i) Bacteriophage.
   j) Colony hybridisation
   k) Electroporation
   l) Transgenic plants applications

PART-'B' LONG QUESTIONS (4X12=48 Marks)

II. Answer all the questions.

2. a) Write about root culture, leaf culture, shoot tip culture.

(or)

b) Define and explain Organogenesis?

3. a) Explain Haploids.

(or)

b) What is cytoplasmic hybridization (or) Cybrids? Mention its procedure, applications.

4. a) What is r-DNA technology? Mention different stages and applications of r-DNA technology.

(or)

b) Define cloning vectors? Write about various cloning vectors.

5. a) Define PCR? Write about its stages and uses.

(or)

b) Write about the gene transformation process through Agrobacterium in higher plants.

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Time: 3 Hrs — VI SEMESTER MODEL PAPER -III — Max. Marks: 80

PART-'A' SHORT QUESTIONS (8X4=32 Marks)

I. Answer any eight from the given questions.

1. a) Sterilization of the medium
   b) Ovule culture
   c)Somatic embryogenesis
   d) Production of stress resistant plant
   e) Cryopreservation
   f) Germplasm conservation
   g) Role of biotechnology in medicine
   h) Applications of.r-DNA.
   i) Ligages.
   j) DNA libraries.
   k) Mibroprojectile
   l) Bt Brinjal

PART-'B' LONG QUESTIONS (4X12=48 Marks)

II . Answer all the questions.

2. a) What is micropropagation? Write about it?

(or)

b) Explain somatic embryogenesis and Zygotic embryogenesis.

3. a) What is tissue culture? Mention its application.

(or)

b) What is somatic hybridization? Write about its procedure, applications.

4. a) What is biotechnology? Explain the role of biotechnology in different fields.

(or)

b) Write about restriction endonucleases , Ligages, Polymerases enzymes in r-DNA technology.

5. a) What are genomic libraries? Write about genomic library, C-DNA library.

(or)

b) Explain Physical gene transfer methods.

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Time: 3 Hrs — **VI SEMESTER MODEL PAPER -IV** — Max. Marks: 80

PART-'A' SHORT QUESTIONS (8X4=32 Marks)

I . Answer any eight from the given questions.

1. a) Micro propagation
   b) Embryo culture
   c) Protoplast culture
   d) Synthetic seeds
   e) Triploids
   f) Cybrids
   g) Role of biotechnology in industry
   h) Polymerases
   i) Larger DNA fragments cloning vectors
   j) PCR
   k) Microinjection
   l) Golden rice

PART-'B' LONG QUESTIONS (4X12=48 Marks)

II . Answer all the questions.

2. a) What is tissue culture? Write about the different stages in tissue culture.

(or)

b) What is callus culture? Explain the process and importance of callus culture.

3. a) Define and explain triploids.

(or)

b) What is cytoplasmic hybridization (or) Cybrids? Mention its procedure, applications.

4. a) What is biotechnology? Explain the role of biotechnology in different fields.

(or)

b) What is r-DNA technology? Mention different stages and applications of r-DNA technology.

5. a) Write about the gene transformation process through Agrobacterium in higher plants.

(or)

b) Explain special importance and useful transgenic plants.