Chapter - 16
Plant Tissue Culture

Robert Hooke discovered the plant cell in 1665, after that in 1839, **Matthias Jacob Schleiden and Theodor Schwann** proposed the cell theory. According to cell theory- "Cell is structural and functional unit of body".

In 1885, **Rudolf Virchow** added to cell theory that "New cells originated from pre-existing cells". On the basis of this fact stated by Rudolf Virchow, cell theory prooved as milestone in the field of biology.

According to Modern Cell theory -

1. Cell is structural and functional unit of body.
2. New cells originated from pre existing cells.
3. All types of metabolic activities take place in cell.
4. In the living beings, hereditary informations are transferred from one generation to next generation through cells.

According to cell theory, each cell of the plant has genetic information for all characters and has the ability to develop the complete plant. This ability of plant cell is called **totipotency**. Totipotency term was given by **Morgan** in 1909. Thus due to totipotency, a plant cell can develop a complete plant in culture. This hypothesis was postulated by German scientist **Haberlandt** in 1902. Totipotency is not present in most of the animals. The culture of plant protoplast, cell, tissue, organ or complete system on chemically known culture medium under sterile and controlled conditions is called **tissue culture**.

German scientist **Gottleib Haberlandt** was first to culture plant tissue in 1902.

Important contribution of various scientist in the field of plant tissue culture is given in Table 16.1.

Important Terminology used in tissue culture

Culture medium- The nutrient medium which consist of chemically defined component and which is used for establishing culture and its multiplication.

Explant- The part of the plant which is used for tissue culture such as meristematic tissue, root, stem, leaf, flower floral part etc.

Callus- The unorganized mass of newly cultured meristematic cells.

Table 16.1 Contribution of various scientist in the field of plant tissue culture

Year	Name of Scientists	Discoveries
1902	Gottlieb Haberlandt	First attempt to culture plant cell on artificial medium
1922	W.J. Robbins and W. Kotte	Culture of plant root and shoot apex successfully (organ culture)
1926	F.W. Went	Discovered the growth hormone Auxin (Indole Acetic Acid)
1939	R.J. Gautheret, P.R. White and P. Nobecourt	Established the long term growth culture medium
1941	Van Overbeek	First time the coconut milk is used in culture medium for cell division
1946	E. Ball	Develop the whole plant from shoot apex Culture (cloning)
1954	W.H. Muir	Develop the isolated single cell culture technique
1955	F. Skoog and Miller	Established the need of kinetin (hormone for cell division) in tissue culture technique
1959	J. Rennert	Develop the embryo from suspension culture of carrot
1959	R.J. Gautheret	Publication of book "Hand book on plant tissue culture"
1960	E.C. Cocking	Separate the protoplast of plant cell by the enzymatic digestion of plant cell wall
1962	T. Murashige and Fo Skoog	Develop the most widely used culture medium (M S medium)
1964	S.Guha Mukherjee and S.C. Maheshwari	Develop the first haploid plant by the culture of pollen grain of Dhatura
1970	J.B. Power	First time successfull demonstration of Protoplast fusion
1978	G. Melchers et al	Development of somatic hybrid pomato by the protoplast fusion of potato and tomato
1983	M.D. Chilton	Development of transgenic plant of tobacco

Protoplast - Cellwall-less plant cell.

Sterilization- The process of destroying or eradication of micro organism.

Surface sterilization- Eradication of micro organism from the surface of the explants used for tissue culture.

Inoculation- The process of transfer of surface sterilized explant on the culture medium.

In Vitro- The process of culture in glassware and plastic ware such as test tube, flask, bottle etc

Clone- Formation of offsprings like mother plant by asexual reproduction methods, are called clones.

Somatic clone- Embryo, which develop from somatic cells.

Artificial/Synthetic seed- Encapsulated somatic embryo.

Embryoids - Embryo like structure which are

formed from somatic cells by *in vitro* method .

Micropropagation- Plant reproduction by tissue culture method.

Necessary resources for plant tissue culture

Tissue culture laboratory should be equipped with modern equipments. As far as possible, this laboratory must not be constructed near the other laboratories which are engaged in conservation of micro organism, insects, plant seeds and other part. During the construction of tissue culture laboratory special care must be taken regarding its design and working place.

In a tissue culture laboratory there must be glassware washing space, culture media preparation room, record room, sterilize transfer chamber, and culture room. The formation of green house and nursery is necessary for hardening, acclimatization and storage of the plantlets which are produced by tissue culture technique.

For an ideal tissue culture laboratory, various equipment and facilities required in different areas have been mentioned in table 16.2.

Table 16.2 Working area and facilities of tissue culture laboratory

S.No.	Working areas	Required facilities and Instruments
1	Glass ware washing space	Necessory facilities and equipments : facility of sufficient water, dishwasher, oven etc.
2	Preparation of culture media	Gas connection, hot plates, heating metals, physical balance and medium digital balance, water purifying system, glass and plastic ware, micro pipette, fume hood, chemical and stock solution, autoclave, refrigerator, centrifuge, magnetic stirrer, pH meter, culture trolley, culture tray etc.
3.	Record related work	Table, chair, almirah, register etc.

Culture medium

The artificial medium in which plant cells, tissue, organs and whole system is cultured is called culture medium. On the basis of experimental needs the culture medium can be liquid or semisolid. Many scientists made efforts to develop suitable culture medium since early stages of the development tissue culture technique.

The culture medium developed by White (White, 1953), B_5 (Gamborg et.al. 1968) and MS (Murashige and Skoog, 1962) are remarkable. Among these, M.S. medium is mostly used in general experiments. The medium which is sterilized and prepared by suitable method is used for tissue culture after cooling.

Steps of Micropropagation- Following steps are used in micropropagation method from selecting explants to completely developed plant.

Zero step- This step is divided in two parts

(I) Selection of explants and pre -treatment- Selection of explant is done by keeping in view the needs and objectives of the experiment. Appropriate explants for various objectives have been shown in table 16.3. Before surface sterilization, the selected explants are washed under the running tap water with the help of Tween-20 by which soil particles and microbes are removed from its surface. This process is known as pre-treatment of explant.

Table 16.3 Appropriate explants according to objectives

S.No.	Objective	Appropriate explant
1.	Cloning	Shoot apex, axillary buds
2.	Virus free plants	Apical meristem
3.	Somatic clonal propagation	Any vegetative part of plant except meristematic tissue
4.	Haploid plant culture	Pollen grain, Anther, Unfertilized egg cell
5.	Protoplast culture	usually leaves
6.	Triploid plant culture	Endosperm
7.	Vegetative embryogenesis	Newly formed plant organ
8.	Callus culture	Any part of the plant

(ii) Surface sterilization of explants - Various type of chemical surface sterilizer like mercuric chloride ($HgCl_2$), Ethanol, silver nitrate ($AgNO_3$), bromine and chlorine water etc. are used in treatment of selective explants in Laminar air flow bench, so that the micro organisms present on the surface of explant can be completely destroyed.

First step : Culture initiation- In this step, the surface sterilized explants are transferred on the culture medium and kept in the culture room. In culture room callus or organogenesis starts.

Second step : Transfer of explants of first step, on the fresh nutrient medium for multiplication

Third step : Regeneration of whole plant

Fourth step : Hardening and acclimatization of plant which developed by tissue culture.

Types of culture : On the basis of experimental objectives, cultures are of the following types -

1. Callus culture - As said earlier for callus culture, any part of the plant can be used as explant. In this type of culture, plant cells undergo an uncontrolled division and produce undifferentiated mass of cells called **callus**. By the use of adequate amount of growth regulators and combination of auxin and cytokinin, a callus can be induced to produce root, shoot, embryoids and complete plantlets.

Regeneration of plants from callus is done by following steps-

i. Organogenesis

ii. Somatic embryogenesis

(i) Organogenesis : The development of plant organs such as root, shoot from group of cells of callus is called **organogenesis**. Development of root from callus is called **rhizogenesis** and development of shoot is called **caulogenesis**. The development of root and shoot depends on auxin and cytokinin ratio, physical state of culture medium, its chemical composition and nature of explants.

Usually the process of rhizogenesis is done on high ratio of auxin is to cytokinin and caulogenesis is on low ratio.

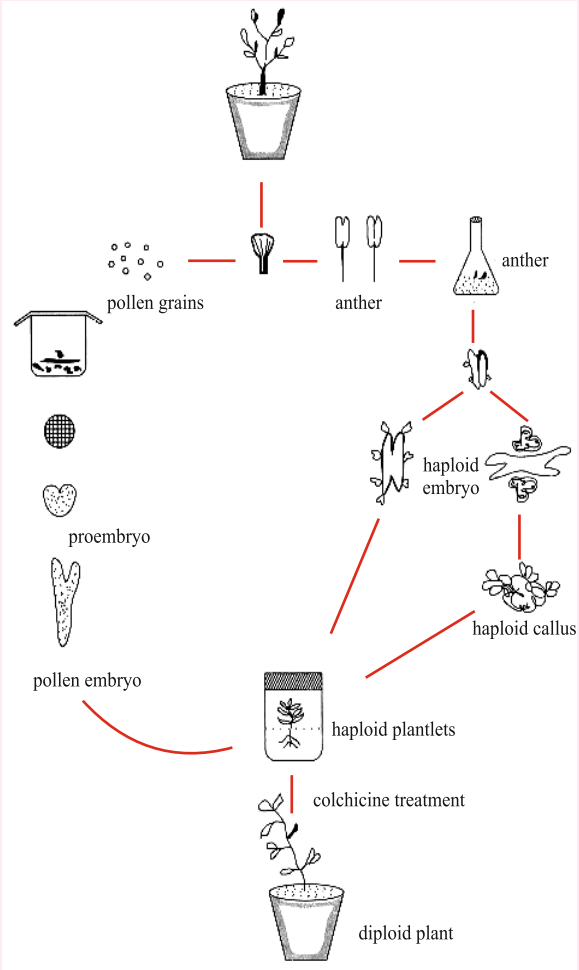

Fig. 16.1 Anther (Culture of haploid plant from pollen grains)

(ii) Somatic embryogenesis- When the embryo is derived from cells of vegetative tissue then this process of embryogenesis is known as **somatic embryogenesis**. The somatic embryo may be haploid or diploid. The haploid or diploid condition of an embryo is dependent on the nature of cells from which it is developed .

First of all, the somatic embryogenesis process was demonstrated by **Reinert** and **Steward** in 1958 . When they cultured the somatic embryo obtained from the callus of carrot (*Daucus carota*) and suspension culture.

2. Organ culture - Any organ such as root, ovary etc of any plant, cultured on culture medium to develop the whole plant is called **organ culture.**

3. Embryo culture- Culture of immature or completely mature embryo of plant is called **embryo culture**. Embryo culture is used for development of immature embryo, growth of mature embryo and inducing it for germination.

Immature embryo culture is also known as **embryo rescue** .

Embryo rescue technique is used for rescue of less developed embryo formed by remote hybridization, from which the rare fertile plants can be developed.

4. Anther and pollen grain culture- First of all,**Shimakure** had done the *in vitro* culture of anther for the study of meiosis in 1943. In 1964, Indian scientist **Shipra Guha Mukherjee** and **Satish Chandra Maheshwari** obtained the haploid plant successfully from the culture of anther and pollen grain of Dhatura plants.

The technique of obtaining haploid plant is used for obtaining pure line in plant reproduction. Maximum number of haploid plants have been developed in Solanaceae family plants.

5. Cell Suspension Culture - Culture of cells in liquid medium is called suspension culture. Suspension culture is used in synthesis and production of bioactive molecules and secondary metabolites at commercial level.

Isolated or single plant cells are usually cultured on aerated liquid culture medium. Cultures are kept on rotating stirrer to keep the cells separate and maintain the oxygen supply in the culture medium.

6. Protoplast - culture- In the process of protoplast culture, first of all sterilized and selected explants are treated with cellulase, hemi-cellulase and pectinase enzymes to remove their cell wall. These cellwall-less plant cells which are called protoplast, are first cultured in liquid culture medium and after that on semi solid medium for developing plants, organogenesis, somatic embryo genesis. Protoplast Culture method is used for **cybrid production**.

Applications and achievements of plant and tissue culture

Plant tissue culture has many application in the field of forestry, agriculture, horticulture and production of medicines.

Some of the important achievements are as follows :-

(1) Micro propagation- The regeneration of plants by tissue culture technique is called **micro propagation**. This technique is used in multiplication of economically important plants, which propagate by natural vegetative reproduction and selection of **'Nobel Plants'**. By this technique , large number of plants can be produced in very less time and space.

By using this technique many species of orchids such as *Cattleya, Cymbidium, Dendrobium, Vanda* and many ornamental plants such as *Gerbera, Chrysanthemum, Carnation, Begonia* can be multiplied on commercial scale.

Many important plants of forestry and horticulture value have been successfully multiplied in many institutes and universities of India.

(2) Regeneration of virus free plants- Many plants are infected by virus and phytoplasma. So virus are easily transmitted into new plants during vegetative propagation. In this condition, it is not possible to get rid of viruses and phytoplasma. Thus, by the apical meristem culture of shoot apex of these plants, virus and phytoplasma free plants can be produced. By virus free plant regeneration technique the virus free plants from plants infested

ith virus such as **Potato Virus S (PVS)** and **Potato Virus X (PVX)** have been produced .

(3) Production of artificial or encapsulated seed- Artificial seeds are produced by embryo-genesis. There are problems in hardening, acclimatization, storage and transportation of plants which are produced by the traditional methods of tissue culture.

To escape from these problems, the somatic embryos and shoot buds are encapsulated with in beads of sodium or calcium alginates. In these beads, nutrients, plant growth regulators, pesticides and antibiotics are also present along with embryo and shoot buds. These seeds germinate like other common seeds .

This type of experiment was initially performed by Murashige in 1977.

(4) Embryo rescue- This technique is used to protect the rare embryos which developed from inter specific hybrids.

(5) Production of androgenic haploids- As stated above androgenic haploids are obtained by anther and pollen grain culture. These haploid plants are used in producing pure line plants in plant reproduction.

(6) Production of triploid plants- Triploid plants are often seedless. The economic value of seed less plants such as apple, banana, orange, pome-granate is enhanced in the field of pomology. The triploid plants can be easily prepared by endosperm culture method of tissue culture technique. Besides the above applications, tissue culture technique is also used in the following fields :-

1. In somatic hybridization
2. In production of somaclonal variation
3. In germ plasm conservation

Method of gene transfer in plants

In the field of agriculture for prospective benefits, the gene transfer and expression of transferred genes in the plant cells, is the modern application of plant tissue culture technique. Any desired foreign gene can be introduced in to the host genome at indefinite or desired location by heredity transformation. Transfer of genes and its expression in the plant cells of higher class, for agriculture, forestry and medicinal field, will be studied under the following heads :-

I. Methods of gene transfer in plants.

II. Genetically modified plants.

I. Methods of gene transfer in plants-

Gene transfer can be done by various methods in plants. Out of these following two methods are very effective and popular.

(A) Vector mediated or Indirect gene transfer

(B) Direct gene transfer

(A) Vector mediated or Indirect gene transfer - In this method, the DNA is transferred by a vector. They are of three types :-

1. Agrobacterium mediated gene transfer- This type of gene transfer is based on **Tumor Inducing Plasmid (Ti Plasmid)**. Ti plasmid is found in ***Agrobacterium tumefaciens*** bacteria which are found in soil and infect the dicot plants. This bacteria induce the uncontrolled cell division in plants, due to which '**gall**' is formed. This gall is called **crown gall**. Uncontrolled cell division in plants and crown gall formation. occurs because of presence of Ti plasmid of *Agrobacterium tumefaciens* bacteria. A copy of Ti plasmid is integrated with the host genome during the process of A. tumefaciens infection in plant cell. The integrated DNA fragment of Ti plasmid into host genome is called T.DNA (Transfer DNA). The infection process of *A. tumefacience* in plants is controlled by Ti plasmid and the gene located on the chromosome (Covalently closed circular DNA ,CCC-DNA) of *Agrobacterium*. On the both ends of transfer DNA ,there is the sequence of direct repeats of 25 base pair nucleotides which is called the left and right end of T- DNA .

The gene to be transferred in the plant cell is introduced or integrated between both these ends. The infection of *Agrobacterium* into plant causes the formation of gall or develop hairy root. These both processes depend on the species of *Agrobacterium*, But in both cases, the host cell get transformed due to the infection of *Agrobacterium*.

In the beginning of 20th century, *Agrobacterium tumifaciencs* and its related species were known as plant pathogen but in the last 3 decades , due to its capabilities of DNA transfer in plant cell, it has been used in the genetic engineering. Due to its use in the genetic engineering, it is called **natural genetic engineer**. Transformation of gene can be detected on the basis of production of amino acid by the genes present in this bacteria. Genetically modified cells produce opines. These opines are of different types which depend on the strain of *Agrobacterium*.

The strain of *A. tumefaciens* produce **octapine and nopalin**, where as the strain of *A. rhizogenes* produce **agropine** and **manopine** opines.

There is possibility of formation of tumor in plants due to Ti plasmid of *A. tumifaciens*. So, for the development of transgenic plants, tumor inducing gene (T-DNA) is seperated and replaced integrated by desired gene and this transformed Ti plasmid is introduced in *Agrobacterium*.

Now the desired gene containing *Agrobacterium* is co-cultured with those tissue of plant or culture in which the desired gene is to be transferred. Generally, the disc or rings of leaves of tomato, tobacco, petunia, rose etc. are used for co-culture because the rings and discs of leaves produce the acetosyrinogone which activate the operons of the plasmid.

Due to activation of these operons, the desired DNA containing Ti plasmid enters in many cells and get integrated with the plant genome. After two-three days of co-culture, the transformed cells are cultured on appropriate culture medium and transformed cells are selected.

This technique of gene transfer can be used only for dicot plants. Ti plasmid is used as gene vector by the scientists of many research institutes.

By the integration of desired and useful gene in T-DNA, useful traits have been developed in many plants such as herbicides, resistance against pathogen and unfavourable condition, increase in nutrient value (Golden rice, rich in Vitamin A), improvement in nitrogen fixation.

Agrobacterium bacteria naturally does not infect the monocot plant but in 1994, Japanese scientist were successful in inducing transformation in the rice plant by Ti plasmid.

2. Virus mediated gene transfer- DNA and RNA virus, both can act as ideal vector for desired genes.

Two virus group having DNA genome, **Calumo virus** and **Gemini virus** have been most widely used for gene transfer.

Retro virus, Lentivirus, Adeno virus etc are also used in genetic engineering for gene transfer.

3. In-planta method- In this technique of gene transfer, **Fieldzman** and **Markakes** (1987) kept genetically modified *Agrobacterium* with seeds of *Arabidopsis* and developed the plant after germination of these seed.

The seeds obtained from these plants which are developed in this way, were germinated on antibiotic free medium and identified the transformed plants. Similarly, the apical meristem of differentiate embryo of germinated seed can also be infected with *Agrobacterium* to produce genetically modified plants. In this method of gene transfer, the genes are directly transfered into plants. Hence, this is called in-planta technique.

(B) Direct methods of gene transfer- In these methods, the transfer of DNA is done directly by some technique. In this, vectors are not used . Gene transfer by *Agrobacterium* is possible only in dicot plants. Gene transfer by Agro infection is not possible in monocot plants which are mainly cereals. In these plants, for quality improvement and transfer of gene for other desired traits, other methods have been developed, in which the biotic vectors are not essential.

Thus, these methods of gene transfer in which the biotic factors such as *Agrobacterium*, virus etc are not needed are called **direct gene** transfer.

In plants, direct gene transfer is done by following methods-

(A) Chemical methods

Some chemicals like Poly Ethylene Glycol (PEG), Poly Vinyl alcohol, calcium phosphate induce the entry of DNA into plant protoplast. PEG

is widely used in chemical methods. In these methods, first plasmid DNA and then after some time 15-25 percent PEG is mixed with protoplast. This quantity of PEG induce the uptaking of DNA by protoplast. There is no harm to protoplast in PEG directed gene transfer method. Thus the transformed prototplasts are cultured on selective medium and transformed protoplast are selected with the help of marker gene. Gene transfer in plants and animals can also be done by **Liposomes, Diethyl Amino Ethyl (DEAE)**, Dextrone proteins etc.

(B) Physical methods of gene transfer-

Gene transfer in plant can also be done effectively by many physical methods including :

1. Gene gun- Gene gun is also known as **particle gun**, **shot gun**, **micro projectile etc**. Gene transfer is also possible into walled plant cell by this device. This technique of gene transfer was first used by **Klein** and co-workers in onion cells for transferring DNA and virus RNA . In this process 1-3 micro meter diameter particles of gold or tungsten coated with desired DNA, which are known as micro particles, are shot at high speed on target cells with the help of micro projectile. These desired DNA coated particles of gold and tungsten penetrate the cell wall and enter the cell, where the desired DNA integrate with DNA of plant cell and forms transgenic DNA. By using this method, gene transfer has been successfully done in soyabean, wheat, paddy, maize, tobacco etc. This technique is used world wide in all type of plants.

2. Electroporation - In this method of gene transfer, the target protoplast, plant cells or tissue are given high voltage pulse due to which, minute temporary pores are formed in plasma membrane. Desired DNA enter into cells through those minute pores. Target cells or tissue are kept in solution containing the desired DNA and high voltage pulse is given, by which this DNA enters into cell first and later enter into nucleus and is integrated with DNA of cell. This method of gene transfer is widely used in monocot plants.

3. Liposome mediated gene transfer- The globular lipid molecule filled with water and desired DNA are used in this method of gene transfer . These DNA aided lipid capsule, first stick to the cell membrane and then conjugate with it. The desired DNA present in these, first enter the cell and then later enter into the nucleus and integrate with the host genome.

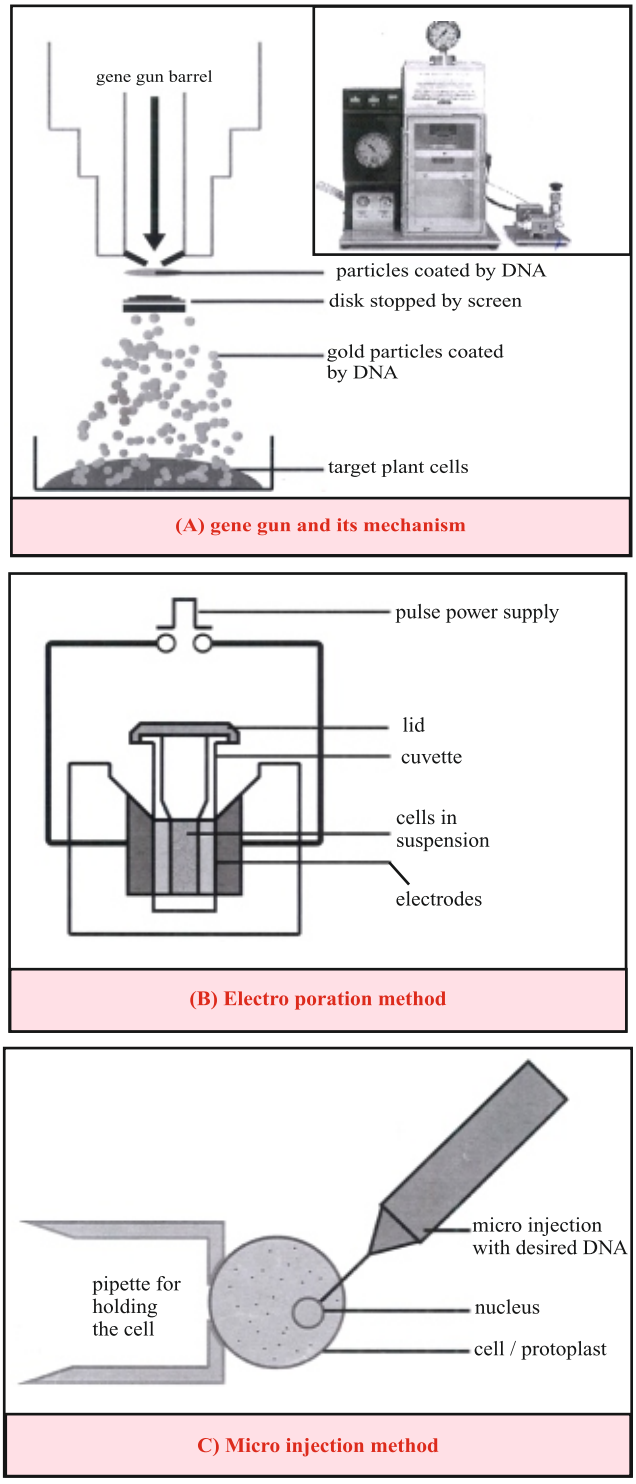

Fig. 16.2 Physical methods of Gene transfer

Liposome directed gene transfer technique that is called **lipofection**, is very effective gene transfer technique in bacteria , animals and plant cells.

4. Microinjection- By this method, the desired DNA is directly injected into cytoplasm or nucleus of plant protoplast or cells with the help of 0.5-1.0 micrometer diameter glass needle or micropippete.

It is a suitable method of gene transfer in isolated protoplast. Besides the above methods of gene transfer, the following methods are also used for gene transfer.

(i) Ladder mediated gene transfer.

(ii) Silicon carbide filament mediated gene transfer.

Selection of genetically transformed cells- Selection of cells genetically transformed, by above methods is an important step in the process of genetic engineering. In this process along with desired gene, marker gene is also introduced in the plant genome. (like antibiotic resistance gene).

After that, they are cultured on culture medium containing suitable supplement and the transformed tissues are separated.

Genetically Modified Plants or Transgenic Plants

Transgenic plants are genetically engineered plants which are produced with additional new qualities by recombinant DNA technology in plant breeding approach. These new qualities are inherited in the offspring, generation after generation. Genetically engineered organisms are also called **Genetically Modified Organisms (GMOs).**

The edible products which are obtained from this type of plants are called **genetically modified foods.**

With the help of genetic engineering the transgenic plant species of monocot and dicot have been developed and they have been experimented outside the laboratory in agricultural fields.

Transgenic plant species can be developed in following steps-

(1) Selection of vector and isolation of transferable gene.

(2) Formation of recombinant plasmid and transformation of *Agrobacterium*.

(3) Transfer of transformed *Agrobacterium* into plant cells.

(4) Selection of transformed plant cells, their culture and regeneration.

(5) Expression of desired gene in developed transgenic plants.

Important Achievements in Agriculture and Medicinal Science Related to Transgenic Plants

1. Insect resistance- *Bacillus thuringiensis* bacteria which is known as **Bt** in short, is naturally found in soil . This bacteria was first of all identified in 1911.

It was identified at that time when larva of 4 moth species were killed by it. It was identified as bio pesticide in America in 1961.

Bt gene is found in this bacteria which coded the insecticidal protein . This gene is also known as **Cry gene**.

This gene is isolated from Bt bacteria and then transferred to cotton and thus the developed cotton variety is known as **Bt cotton** or **Killer cotton**. This cotton is resistant against Boll worm insect.

2. Herbicide resistance- Generally, most of the herbicides, kill the plants by inhibiting the synthesis of essential amino acids. Such as like **Glyphosate** herbicide suppress the activity of enzyme **5-Enol Pyruvil Shikimate-3-Phospho Synthetase (EPSPs)** which are used in synthesis of aromatic amino acids. Gene related to more production of EPSPs enzyme, has been transferred to plants to reduce the effect of glyphosphate. This type of transgenic plants have been developed in tomato, *Petunia* etc. By the genetic engineering technique, transgenic plants of crops and economically important stress resistance have been developed.

3. Development of male sterility- Sterile male plants are very important in the hybridization experiment in plant breeding. Due to development of sterile male lines, time, money and labour is not

wasted in the processes like emasculation. **Barnase gene** obtained from *Bacillus amyloliquefaciens* can be integrated with the genome of rape seed with the help of *Agrobacterium* to get male sterile plants. Barnase gene destroy the tapetal RNA of anther which causes the development of male sterile plants.

4. Delay fruit ripening- Flavr Savr is a genetically engineered variety of transgenic tomato produced in America .This variety of tomato persist for a long period as compared to normal tomato. This variety of tomato is produced by reducing the level of enzyme **poly galacturonase** which degrade the cell wall. This enzyme is responsible for fruit ripening.

5. Transgenic plant bioreactor- Genetically modified plants are used as bio reactor. Various type of chemicals are produced.

In these type of transgenic plants. Hence, this area of biotechnology is called **molecular farming**.

Following plants are developed in this way-

1. Golden rice (Vitamin -A)
2. Super potato
3. Seed protein quality
4. Edible vaccines
5. Important medicines
6. Bio degradable plastics
7. Metabolic engineering

Transgenic plants with transformed floral colours

Floral business is important in Netherland. Floral colour of *Petunia* is not saffron but the scientist of Netherland developed a variety of *Petunia* with saffron colour flower by the transfer of gene into *Petunia* which was isolated from maize. Similarly with the help of genetic engineering, blue rose, blue carnation and blue tulip have been developed .

Adverse effect of transgenic plants on environment, ecology and human beings- There are the following dangers from transgenic plants and plants developed by genetic engineering .

- Transfer of transferred gene naturally to same species crops and near by wild species plants.
- Possibilities of emerging of new epidemic disease in highly resistant crops.
- Possibilities of allergy in users by usage of products of transgenic plants.
- Possibilities of entry of genes of animals , microbes , viruses etc. into important plants.
- Danger of destruction of natural biodiversity and section of new variety by nature.
- Effect on agriculture practice and imbalance in ecosystem.

Future prospects of transgenic plants-

Despite the above mentioned adverse effects, important traits are being transferred into plants by scientist to fulfill the requirement of ever increasing population by genetic engineering technique. For human culture and society, transgenic plants are being developed regularly.

Out of which some important possibilities are-

1. Transfer of nitrogen fixing gene (nif gene) for atmospheric nitrogen fixation in cereal plants such as wheat, rice etc.
2. Recombination of nuclear and chloroplast gene from best source to increase the efficiency of photosynthesis and converting C_3 plants into C_4 plants (C_4 plants are photosynthetically more efficient)
3. Development of eco- friendly plants. The plants that have capacity to degrade the pollutants of air, soil, water, are called eco friendly plants.
4. Desired changes in the gernination and anthesis time of vegetables and fruits to get rid of dependence of plants on seasons.

Important Points

1. The capacity of plant cell due to which it can divide and differentiate to from a whole plant of which it is a part is called toti potency.
2. The first effort for plant tissue culture was made by Gottlieb Haberlandt in 1902.
3. Chemically known medium which is used for tissue culture is called culture medium.

4. Cell wall less plant cell is known as protoplast.
5. First of all, Shipra Guha Mukherjee and Satish Chandra Maheshwari developed the haploid plant of Dhatura.
6. The process of micro propagation is completed in 5 steps :- Zero step, First step, Second step, Third step, and Fourth step.
7. The embryo developed from somatic cell of plant, somatic embryo and encapsulated embryo are called artificial seed.
8. Virus free plants can be developed by shoot apex culture.
9. Triploid plants are developed by endosperm culture.
10. Indirect gene transfer in plants is done by *Agrobacterium*.
11. For the delayed ripening of fruit of tomato 'flavrSavr' and 'golden rice' with vitamin-A transgenic plants have been developed.

Practice Questions

Multiple Choice Questions-

1. Who has been credited for producing haploid plants from anther culture for first time-
   (a) Johari and Maheshawari
   (b) Haberlandt
   (c) P.R. White
   (d) Guha and Maheshwari
2. Disease free plants from infected plants is developed-
   (a) By embryo culture
   (b) By root culture
   (c) By anther culture
   (d) By shoot apical meristem culture
3. Who is known as father of plant tissue culture-
   (a) Robert Hook (b) Haberlandt
   (c) Steward (d) Kocking
4. Ti plasmid is found
   (a) In *A. tumifaciens*
   (b) In *A. rhizogenes*
   (c) In *E. coli*
   (d) In *Bacillus thuringiensis*
5. Who is credited for obtaining protoplast by enzymatic degradation of cell wall-
   (a) T. Murashige (b) E. Ball
   (c) F.W. Went (d) E.C. Kocking
6. Which of the following is taken as explants for triploid culture in plant-
   (a) Shoot apex (b) Embryo
   (c) Endosperm (d) Anther
7. Indirect gene transfer is done by-
   (a) Gene gun
   (b) Electropration
   (c) Micro injection
   (d) Agrobacterium
8. Insect resistant Bt gene is found in which of the following-
   (a) *Bacillus subtilis*
   (b) *Bacillus thuringiensis*
   (c) *Bacillus anthresis*
   (d) *Pseudomonas citri*
9. In 'golden rice' gene for which of the following has bee transfered -
   (a) Vitamin A (b) Vitamin C
   (c) Vitamin D (d) Vitamin B
10. The quality of 'flavr savr' tomato is-
    (a) Drought resistant
    (b) High salt concentration resistance
    (c) rigid fruit wall
    (d) All of the above
11. Bt gene containing cotton is called
    (a) Aseptic cotton (b) Hairy cotton
    (c) Golden cotton (d) Killer cotton

Very short answer Questions-

1. Define totipotency?

2. What is artificial seed?
3. What is Callus?
4. What is the significance of haploid plant culture?
5. Which are the culture medium used for plant tissue culture?
6. What do you understand by micro propagation?
7. Write the name of three methods of direct gene transfer?
8. What do you understand by indirect gene transfer?
9. What are the main components of artificial (encapsulated) seed?
10. Define the following
    (i) Culture medium
    (ii) Protoplast
    (iii) Explant
    (iv) Transgenic plant

Short Answer Questions

1. Explain the method of micro injection in gene transfer.
2. Mention any two insect resistant plants.
3. Write the different steps of tissue culture.
4. What is the difference between a normal embryo and somatic embryo?
5. Mention the name of methods of micro propagation and its uses.
6. What is embryo rescue technique? Mention its use.
7. Comment on Ti plasmid.
8. What do you understand by tissue culture?

Essay Type Questions

1. Write a short note on history of plant tissue culture.
2. What is transgenic plant? Describe about its development and utility.
3. Explain about indirect (vector mediated) methods of gene transfer.
4. Briefly explain the different phases of tissue culture.
5. Explain the methods of gene transfer with the help of labeled diagrams.
6. Explain different methods of micro propagation.
7. What are the different components of culture medium? Mention them.

Answer Key

1. (d) 2. (d) 3. (b) 4.(a) 5. (d)
6. (c) 7. (d) 8. (b) 9. (a) 10. (c)
11. (d)