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# Cell Culture Technologies-A Short Review

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***Abstract:*** *Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment.The cells may be removed from the tissue directly and disintegrated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line.The term "cell culture" now refers to the culturing of cells derived from multicellular eukaryotes, especially animal cells, in contrast with other types of culture that also grow cells, such as plant tissue culture, fungal culture, and microbiological culture (of microbes)[1].*

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## I. Introduction

Cells, the fundamental unit of life, are an incredibly useful tool in biological research. They serve as an imperative model system for understanding physiological processes and screening of toxic or therapeutic compounds for use in medical treatments. In addition, cells play a major role in functional enzyme, growth factor and vaccine production, along with a multitude of other uses. In order to accomplish above mentioned practices, cells must first be cultured in an environment outside from the organism they originate[2]. This is the process of cell culturing. To elaborate, cell culturing involves maintaining cells of multi-cellular organisms outside of their original body under precise conditions.

## II. Importance Of Cell Culture

*In Vaccine Research*

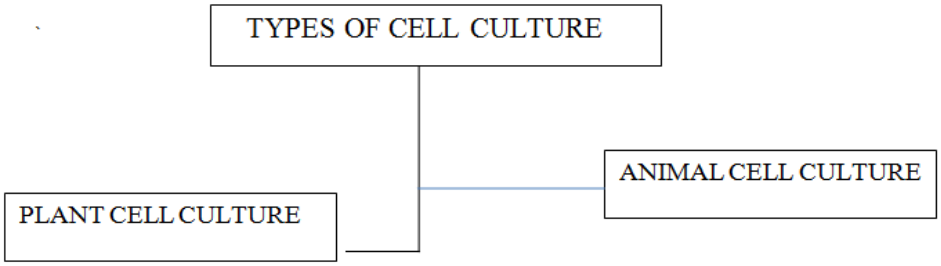
Cell culture has a diverse range of uses as cultured cells are used by cell biologists, biomaterials scientists, clinicians and regulatory authorities, among others . One of the most important uses of cell culture is in the research and production of vaccines. The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce vaccines for many other diseases. Early in the 1930s and 40s, researchers had to use live animals to grow poliovirus, but with the advent of cell culturing, they were able to achieve much greater control over virus production and on a much larger scale . This allowed researchers to study them more closely and eventually develop vaccines and various treatments.[3]

*In Protein Therapeutics*

Another important use of cell lines is to express different types of proteins in mammalian cells . Initially, E. coli was the primary organism used for producing proteins, but with the need to create properly folded proteins with the proper post translational modifications, the focus shifted to using eukaryotes instead . Starting from the 1970s and 80s, proteins like interferon and antibodies have been successfully created via cell culture. various cytokinins and growth factors can be acquired from cultures, and looking at the structure and activity of these proteins helps us understand their role in the organism's body as well.

*In Cancer Research*

Cancer is one of the leading causes of death around the world and millions of dollars are being used for cancer research to find treatments and cures. Cell culture is crucial here as well, as normal cells can be transformed into cancer cells by methods including radiation, chemicals and viruses . These cells can then be used to study cancer more closely and to test new treatments[4]




**PLANT CELL/TISSUE**

*Gottlieb Haberlandt* first initiated tissue culture technique in 1902.Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs especially on artificial medium in suitable containers under controlled environmental conditions.The part which is cultured is called explant, i.e., any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell/explant is called cellular toti-potency. In fact, the whole plant can be regenerated from any plant part (referred to as explant) or cells.

**Hormones used in Plant Tissue Culture:**

1. Auxins neoline (Indole-3-acetic acid, Indole-3-butyric acid, Potassium Salt— Naphthalene acetic acid 2, 4-Dichlorophenoxyacetic acid p-Chloro-phenoxy acetic acid)
2. Cytokinins (6-Benzylaminopurine, 6-Dimethylallylaminopurine (2ip), Kinetin)
3. Gibberellins (Gibberellic Acid)
4. Abscisic Acid (ABA) (Abscisic Acid)
5. Polyamines (Putrescine, Spermidine)

**Environmental Conditions:**

There are three important aspects
(i) nutrient medium,
(ii) aseptic conditions and
(iii) aeration of the tissue

**1. Nutrient Medium:**

The composition of plant tissue culture medium can vary depending upon the type of plant tissues or cell that are used for culture. A typical nutrient consists of inorganic salts (both micro and macro elements), a carbon source (usually sucrose), vitamins (e.g., nicotonic acid, thiamine, pyridoxine and myoinositol), amino acids (e.g., arginine) and growth regulators (e.g., auxins like 2,4-D or 2,4-dichlorophenoxyacetic acid and cytokinins such as BAP = benzlaminopurine and gibberellins). Other compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice, etc. may be added for specific purposes.

Plant hormones play important role in growth and differentiation of cultured cells and tissues. An optimum pH (usually 5.7) is also very important. The most extensively used nutrient medium is MS medium which was developed by *Murashige* and *Skoog* in 1962. Usually a gelling agent agar (a polysaccharide obtained from a red algae Gelidium amansi) is added to the liquid medium for its solidification.[5]

**2. Aseptic Conditions (Sterilization):**

Nutrient medium contains ample sugar which increases growth of microorganisms such as bacteria and fungi. These microbes compete with growing tissue and finally kill it. It is essential to maintain aseptic conditions of tissue culture. Thus sterilization means complete destruction or killing of microorganisms so that complete aseptic conditions are created for in vitro culturing.

**3. Aeration of the Tissue:**

Proper aeration of the cultured tissue is also an important aspect of culture technique. It is achieved by occasionally stirring the medium by automatic shaker.

**Plant Material—the Explant:**

Any part of a plant taken out and grown in test tube under sterile conditions in special nutrient media is called explant.[6]

**Methods of Plant Tissue Culture:**

Plant tissue culture includes two major methods:
(A) Type of in vitro growth-*callus and suspension cultures*.

(B) Type of explant— single cell culture, shoot and root cultures, somatic embryo culture, meristem culture, anther culture and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary culture, etc.[7]

**Types of Plant Tissue Culture:**

**Callus and Suspension Cultures:**

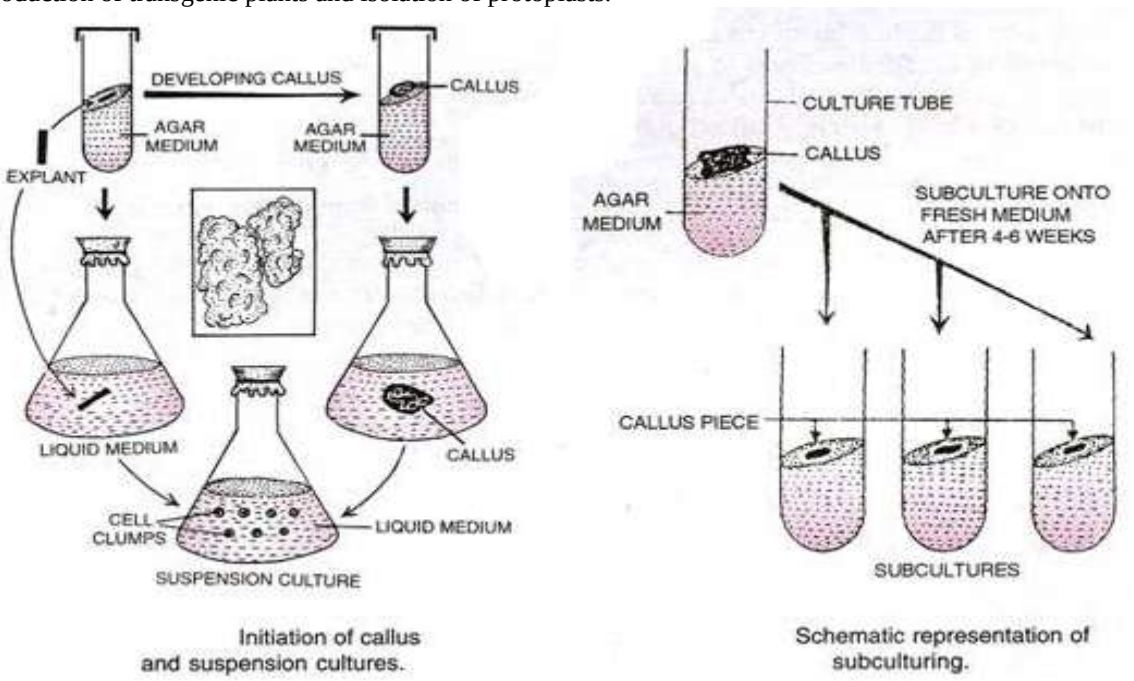
In callus culture, cell division in explant forms a callus. Callus is irregular unorganised and undifferentiated mass of actively dividing cells. Darkness and solid medium gelled by agar stimulates callus formation. The medium ordinarily contains the auxin, 2,4-D, (2, 4- dichlorophenoxy acetic acid) and often a cytokinin like BAP (Benzyl aminopurine). Both are growth regulators. This stimulates cell divison in explant. Callus is obtained within 2-3 weeks.

A suspension culture consists of single cells and small groups of cells suspended in a liquid medium. Usually, the medium contains the auxin 2,4-D. Suspension cultures must be constantly agitated at 100-250 rpm (revolutions per minute). Suspension cultures grow much faster than callus culture.[8]

**Sub culturing:**

If tissue cultures are kept in the same culture vessel, they die in due course of time. Therefore, cells/tissues are regularly transferred into new culture vessels containing fresh media. This process is called *sub culturing*. It is important to note that during subcultureonly a part of the culture from a vessel is transferred into the new culture vessel.

The callus and suspension cultures may be used to achieve cell biomass production, regeneration of plantlets, production of transgenic plants and isolation of protoplasts.




Digramatic Representation of Sub culturing

**Single Cell Culture:**

As mentioned earlier, cells derived from a single cell through mitosis constitute a clone and the process of obtaining clones is called cloning (asexual progeny of a single individual make up a clone). There are two popular techniques for single cell culture.[9]

**Bergmann's Plating Technique:**

This is widely used technique. The cells are suspended in a liquid medium at a cell density that is twice the desired density in the plate. Sterilized agar (Ca 1%) medium is kept in a water bath at 35°C. Equal volumes of the liquid and agar media are mixed and spread in Ca 1 mm thick layer in a petridish. The cells remain embedded in the soft agar medium which is observable under a microscope. When large colonies develop they are isolated and cultured separately.

**Filter Paper Raft Nurse Tissue Technique:**

Single cells are placed on small pieces (8×8 mm) of filter paper, which are placed on top of callus cultures several days in advance. This allows the filter papers to be wetted by the callus tissues. The single cells placed

on the filter paper derive their nutrition from the callus. The cells divide and form macroscopic colonies on the filters. The colonies are isolated [10]and cultured.

**Shoot and Root Cultures:**

Shoot culture is promoted by a cytokinin like BAR However, root culture is promoted by an auxin like NAA (naphthalene acetic acid). The shoot and root cultures are generally controlled by auxin-cytokinin balance. Usually, an excess of auxin promotes root culture, whereas that of cytokinin promotes shoot culture. Roots culture from the lower end of these shoots to give complete plantlets.[11]

**Somatic Embryo Culture:**

A somatic embryo develops from a somatic cell. The pattern of development of a somatic embryo is comparable to that of a zygotic embryo. Somatic embryo culture is induced by a high concentration of an auxin, such as 2,4-D. These embryos develop into mature embryos. Mature somatic embryos or embryoids germinate to give complete [12]plantlets.

**Establishment in the Field:**

The plantlets are removed from culture vessels and established in the field. This transfer is done by specific procedures called hardening. During hardening, plantlets are kept under reduced light and high humidity. Hardening procedures make the plantlets capable of tolerating the relatively harsher environments outside the culture vessels.

**Endosperm Culture:**

Tissue culture methods are also used for culturing endosperm. It is unique because it supplies nutrition to the developing embryo. It is also triploid in its chromosome constitution. Triploid plants are used for the production of seedless fruits (e.g., apple, banana etc.). The technique of endosperm culture involves the following:

(i) The immature seeds are dissected under aseptic condition. Endosperms along with embryos, are excised. Sometimes, mature seeds can also be used.

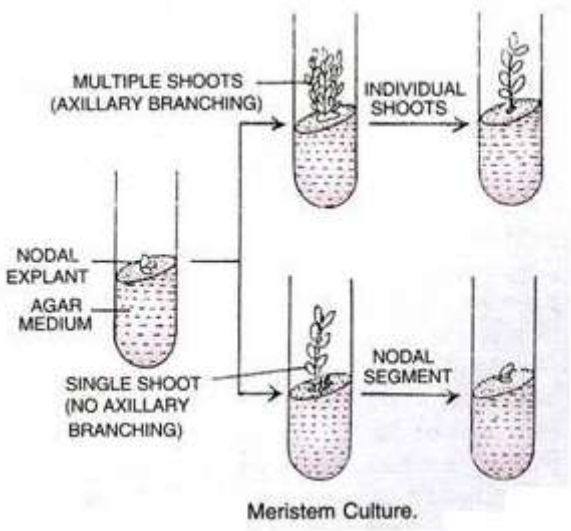
(ii) The excised endosperms are cultured on a suitable medium and embryos are removed after initial growth.

(iii) The initial callus phase is developed.

(iv) The shoots and roots may develop and complete triploid plants are formed for further use.[13]

**Meristem Culture:**

Meristem is a localized group of cells, which are actively dividing and undifferentiated but ultimately giving rise to permanent tissue. Although the plant is infected with a virus, yet the meristem is free of virus. Therefore, meristem can be removed and grown in vitro to obtain virus free plants. Cultivation of axillary or apical shoot meristems is called meristem culture. The apical or axillary meristems are generally free from virus. Meristem culture involves the development of an already existing shoot meristem and subsequently, the regeneration of adventitious roots from the developed shoots.[14]

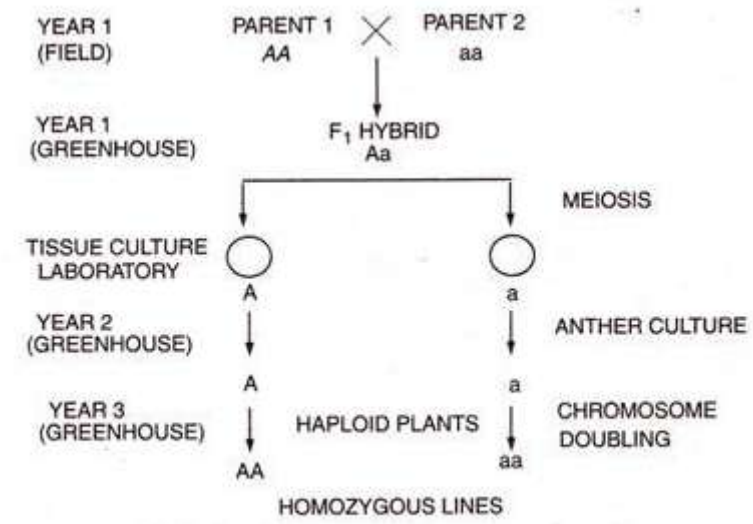



Meristem Culture

It usually does not involve the regeneration of a new shoot meristem. The explants commonly used in meristem culture are shoot tips and nodal segments. These explants are cultured on a medium containing a cytokinin (generally BAP). The plantlets thus obtained are subjected to hardening and, ultimately, established in the fiddi Meristem culture is carried out in Potato, Banana, Cardamom, Orchids (protocorm stage), Sugarcane, Strawberry, Sweet Potato, etc. It is used in (i) Production of virus-free plants like potato, sugarcane, banana and apple, (ii) Germplasm conservation, (iii) Production of transgenic plants, (iv) Rapid clonal multiplication

**Anther Culture and Haploid Production:**

An individual/cell having the chromosome number found in the gametes of the species is called haploid. Formation of haploid is called haploid production. Thus haploid individuals arise from the gametes. A haploid has only one copy of each chromosome. Haploids are sterile and of no direct value[15]




Production of homozygous lines using anther culture.
The two parents are shown to differ for only one gene, *i.e.*, AA and aa.

When the chromosome number of a haploid plant is doubled, the plants of normal chromosome number for particular species are obtained. These plants are homozygous and are produced in 2-3 years. The chromosome number of these haploid plants is doubled by using colchicine to obtain homozygous plants.

In nature, haploid plants originate from unfertilized egg cells, but in laboratory, they can be produced from both male and female gametes. Anther is the part of the flower of Angiosperms producing pollen (microspores), borne at the end of the stamens and usually consisting of four sporangia. When anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture.

The technique was developed by *Guha* and *Maheshwari* (1964) who cultured mature anthers of Datura innoxia. It is highly useful for the improvement of many crop plants. [16]It is also useful for immediate expression of mutations and quick formation of purelines. This technique was first used in India to produce haploids of Datura. In many plants, haploids are also produced by culturing unfertilized ovaries/ovules. Sometimes, pollen grains are separated from anthers and cultured on suitable medium.

**Embryo Culture:**

Culturing young embryos on a nutrient medium is called embryo culture. Young embryos are obtained from the developing seeds. The embryos complete their development on the medium and grow into seedlings. In general, older embryos are more easily cultured in vitro than young embryos.[17]

**Embryo culture is useful as follows:**

(i) Orchid seeds do not have any form of stored food. Embryos of such seeds can be cultured to obtain seedlings and maximum seedling formation can be achieved. Embryo culture in orchids can be applied for rapid clonal propagation.

(ii) In certain species, inhibitors present in the endosperm or seed coat make the seed dormant. Such embryos can escape dormancy by culturing on a suitable medium.

(iii) In certain hybrid seeds developed after interspecific crosses, the endosperm degenerates at an early stage and the young embryo is left with no food, consequently it also dies. Such young embryos can be excised from the seeds and cultured on the nutritive medium. Getting nutrition, they develop into seedlings which can be transplanted in the field.

(iv) A popular example includes hybridization of barley and wheat with *Hordeum bulbosum* leading to the production of haploid barley and haploid wheat respectively. Haploid wheat plants have also been successfully obtained through culture of hybrid embryos from wheat x maize crosses.

**Ovule Culture:**

Ovule culture technique is utilized for raising hybrids which normally fail to develop due to the abortion of the embryos at an early stage. Ovules can easily be excised from the ovary and cultured on the basal medium. The loss of a hybrid embryo due to premature abscission of fruits may be prevented by ovule culture. In some cases, addition of fruit/vegetable juice increase the initial growth.

**Ovary culture:**

Ovary culture technique has also been successfully employed to raise interspecific hybrids between sexually incompatible species, *Brassica campestris* and *B. oleracea.* Ovaries are excised from the flowers and cultured at the zygote or two-celled proembryo stage for obtaining normal development on culture medium. Sometimes coconut milk when used as a supplement to the medium promote formation of fruits that are larger than those formed in vivo (within the living organism). In Anthem, addition of kinetin in the medium caused polyembryony which gave rise to multiple shoots.

**Micro propagation:**

Micropropagation is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees by using small sized explants. Because of minute size of the propagules in the culture, the propagation technique is named as mircopropagation. This method of tissue culture produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown.

The genetically identical plants developed from any part of a plant by tissue culture/micropropagation are called somaclones. The members of a single somaclone have the same genotype. This micropropagation is also known as somaclonal propagation. It is the only process adopted by *Indian plant biotechnologists* in different industries mainly for the commercial production of ornamental plants like lily, orchids, Eucalyptus, Cinchona, Blueberry, etc. and fruit trees like tomato, apple, banana, grapes, potato, citrus oil palm, etc.

There are four defined steps in micro propagation method. These are:
(i) Initiation of culture from an explant like shoot tip on a suitable nutrient medium.
(ii) Shoot formation multiple shoots formation from the cultured explant.
(iii) Rooting of shoots rooting of in vitro developed shoots.
(iv) Transplantation the hardening of tissue culture raised plants and subsequent transplantation to the field.[18]

**Advantages of Micro propagation:**

These are as follows:
1. It helps in rapid multiplication of plants.
2. A large number of plantlets are obtained within a short period and from a small space.
3. Plants are obtained throughout the year under controlled conditions, independent of seasons.
4. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.
5. It is an easy, safe and economical method for plant propagation.
6. In case of ornamentals, tissue culture plants give better growth, more flowers and less fall-out.
7. Genetically similar plants (somaclones) are formed by this method. Therefore, desirable characters (genetope) and desired sex of superior variety are kept constant for many generations.
8. The rare plant and endangered species are multiplied by this method and such plants are saved.

**Regeneration of Plantlets:**

**1. Preparation of Suitable Nutrient Medium:**

Suitable nutrient medium as per objective of culture is prepared and transferred into suitable containers.

**2. Selection of Explants:**

Selection of explants such as shoot tip should be done.

**3. Sterilisation of Explants:**

Surface sterilization of the explants by disinfectants and then washing the explants with sterile distilled water is essential.

**4. Inoculation:**

Inoculation (transfer) of the explants into the suitable nutrient medium (which is sterilized by filter-sterilized to avoid microbial contamination) in culture vessels under sterile conditions is done.

**5. Incubation:**

Growing the culture in the growth chamber or plant tissue culture room, having the appropriate physical condition (i.e., artificial light; 16 hours of photoperiod), temperature (-26°C) and relative humidity (50-60%) is required.

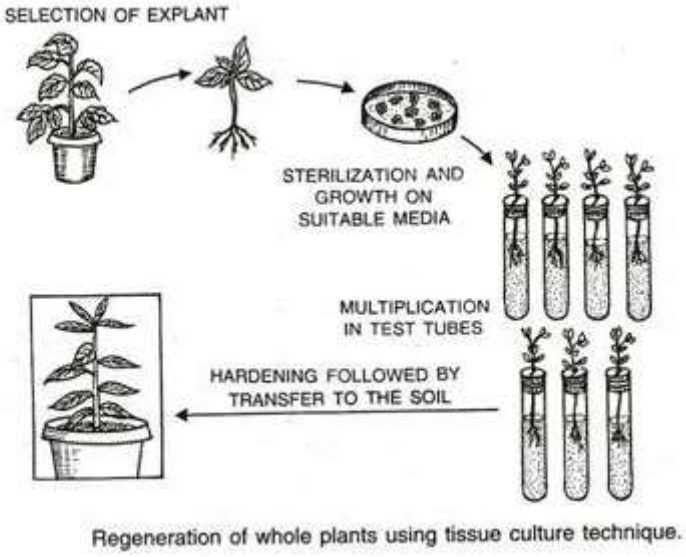
**6. Regeneration:**

Regeneration of plants from cultured plant tissues is carried out.

**7. Hardening:**

Hardening is gradual exposure of plantlets to an environmental conditions.

**8. Plantlet Transfer:**
After hardening plantlets transferred to the field conditions following acclimatization (hardening) of regenerated plants.




Acclimatization of Regenerated Plants

**Protoplast Culture and Somatic Hybridisation:**

When a hybrid is produced by fusion of somatic cells of two varieties or species, it is known as somatic hybrid. The process of producing somatic hybrids is called somatic hybridisation. First, the cell wall of the plant cells is removed by digestion with a combination of pectinase and cellulase. The plant cells without cell wall are called protoplasts.[19]

The protoplasts of the two plants are brought together and made to fuse in a solution of polyethylene glycol (PEG) or sodium nitrate. The fusion of protoplasts with the help of chemicals is called chemo-fusion. Fusion of protoplasts with the help of high voltage pulse is known as electro-fusion. The fusion of protoplasts not only involves the fusion of their cytoplasm but also their nuclei. The fused protoplasts are allowed to grow on culture medium. Soon they develop their own walls when they are called somatic hybrid cells.

The hybrid cells give rise to callus. Callus later differentiates into new plant which is somatic hybrid between two plants. Somatic hybrids in plants were first obtained between two species of Tobacco (*Nicotiana glauca and N. langsdorfit)* by *Carlson etal* in 1972. Successful somatic hybrids have also been got from different species of Brassica, Petunia, and Solanum.

Pomato is somatic hybrid between Potato and Tomato that belong to two different genera and Bomato is somatic hybrid between Brinjal and Tomato. Somatic hybrids are also produced between rice and carrot. The hybrid plant bears both fruits and tubers of the two parents.[20]

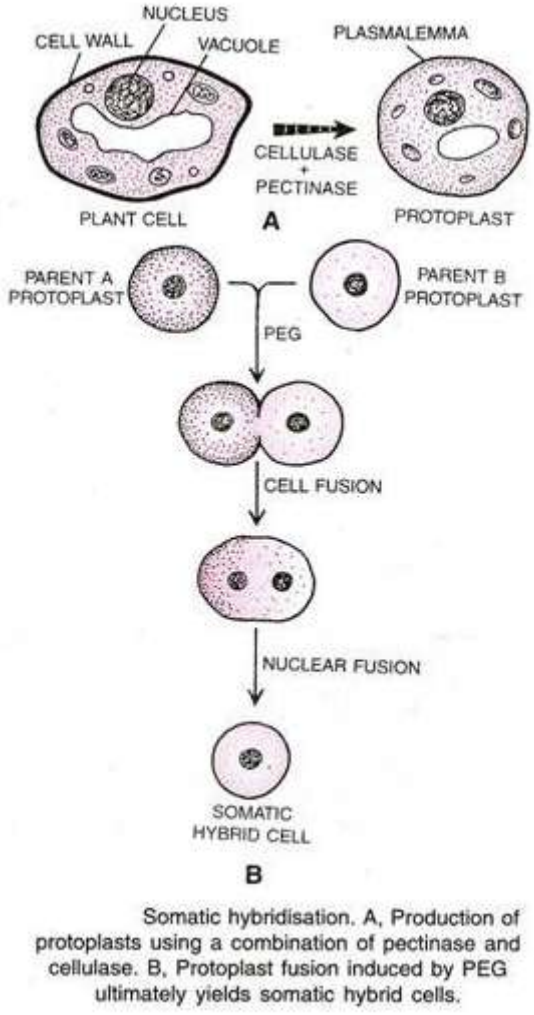
(a) Protoplast technology has opened up avenues for development of hybrids of even asexually reproducing plants.

(b) There is a distinct possibility of development of new crop plants, e.g., Pomato.

(c) Somatic hybrids may be used for the production of useful allopolyploids.

(d) Genetic manipulations can be carried out more rapidly when plant cells are in protoplast state. New genes can be introduced (e.g., male sterility, herbicide resistance). Mutations will be easier.

If we conclude, plant tissue culture is a broad term used to define different types of in vitro plant culture. It may be recognized in the following types. Each type can result in a whole plant. (1) Callus culture — culture of differentiated tissue from an explant that dedifferentiates. (2) Cell culture — culture of cells or cell aggregates (small clumps of cells) in liquid medium. (3) Protoplast culture — culture of plant cells with their cell walls removed. (4) Embryo culture — culture of isolated embryos. (5) Seed culture — culture of seeds to generate plants. (6) Organ culture — culture of isolated plant organs such as anthers, roots, buds and shoots.




Somatic hybridisation. A, Production of protoplasts using a combination of pectinase and cellulase. B, Protoplast fusion induced by PEG ultimately yields somatic hybrid cells.

Somatic Hybridization

**Artificial Seeds:**

There are many plants which neither have seeds nor produce a small quantity of seeds. To overcome this problem the concept of artificial seeds has become popular, where somatic embryos are encapsulated in a suitable matrix composed of sodium alginate, along with substances like mycorrhizae, herbicides, fungicides and insecticides. The technique involved in the production of artificial seeds is based on cellular totipotency and somatic embryogenesis.[21]

An artificial seed is a bead of gel containing a somatic embryo (or shoot bud) and the nutrients, growth regulators, antibiotic, etc. needed for the development of a complete plantlet. Artificial seeds may be produced using one of the following two ways: desiccated systems and hydrated systems. In the desiccated systems the somatic embryos (SEs) are first hardened to withstand desiccation and then are encapsulated.

In the hydrated systems, the beads become hardened as calcium alginate is formed, after about 20-30 minutes the artificial seeds are removed, washed with water and used for planting. Hydrated artificial seeds become dry rapidly in the open air. Therefore, hydrated artificial seeds have to be planted soon after they are produced.

In India, this technique of synthetic seeds is being done for sandalwood and mulberry at BARC (Bhaba Atomic Research Centre), Mumbai. Advantages (i) They can be directly sown in the soil like natural seeds, (ii) They can be stored upto a year without loss of viability, (iii) They are easy to handle, and useful as units of delivery.

The only disadvantage of artificial seeds is the high cost of their production.

**Practical Applications of Plant Tissue Culture:**

The use of plant cells to generate useful products and/or services constitutes plant biotechnology. In plant biotechnology, the useful product is a plantlet. The plantlets are used for the following purposes.

**1. Rapid Clonal Propagation:**
A clone is a group of individuals or cells derived from a single parent individual or cell through asexual reproduction. All the cells in callus or suspension culture are derived from a single explant by mitotic division. Therefore, all plantlets regenerated from a callus/suspension culture generally have the same genotype and constitute a clone. These plantlets are used for rapid clonal propagation. This is done in oil palm.

**2. Somaclonal Variation:**
Genetic variation present among plant cells of a culture is called somaclonal variation. The term somaclonal variation is also used for the genetic variation present in plants regenerated from a single culture. This variation has been used to develop several useful varieties.

**3. Transgenic Plants:**
A gene that is transferred into an organism by genetic engineering is known as transgene. An organism that contains and expresses a transgene is called transgenic organism. The transgenes can be introduced into individual plant cells. The plantlets can be regenerated from these cells. These plantlets give rise to the highly valuable transgenic plants.[22]

**4. Induction and Selection of Mutations:**
Mutagens are added to single cell liquid cultures for induction of mutations. The cells are washed and transferred to solid culture for raising mutant plants. Useful mutants are selected for further breeding. Tolerance to stress like pollutants, toxins, salts, drought, flooding, etc. can also be obtained by providing them in culture medium in increasing dosage. The surviving healthy cells are taken to solid medium for raising resistant plants.

**5. Resistance to Weedicides:**
It is similar to induction of mutations. Weedicides are added to culture initially in very small concentrations. Dosage is increased in subsequent cultures till the desired level of resistance is obtained. The resistant cells are then regenerated to form plantlets and plants

**APPLICATIONS OF PLANT CELL CULTURE:**
1. Micro Propagation
2. Clonal Propagation
3. Production of Genetically Variable Plants
4. Plant Pathology and Plant Tissue Culture
5. Plant Breeding, Plant Improvement and Plant Tissue Culture
6. Production of Useful Bio-chemicals
7. Preservation of Plant Genetic Resources or Gene Conservation Banks
8. Importance of Tissue Culture in Biotechnology

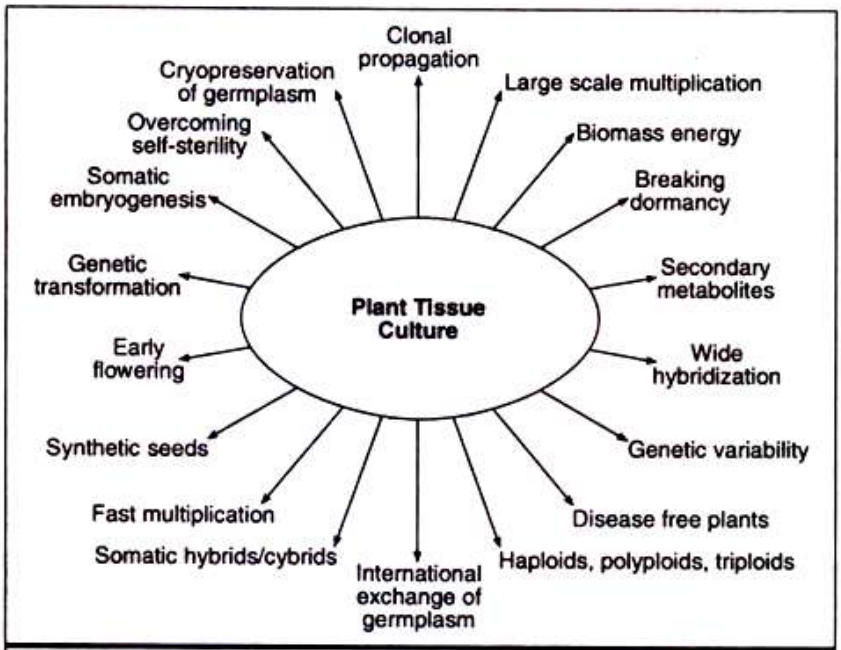



**Fig. 16.6:** Plant improvement through tissue culture technology

*Diagramatic Representation of Plant Tissue Culture*

## III. Animal Cell Culture

**History of Animal Cell Culture:**

Although animal cell culture was first successfully undertaken by *Ross Harrison* in 1907, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists. First, there was the development of *antibiotics* that made it easier to avoid many of the contamination problems that plagued earlier cell culture attempts.[23] Second was the development of the techniques, such as the use of Trypsin to remove cells from culture vessels, necessary to obtain continuously growing cell lines (such as HeLa cells). Third, using these cell lines, scientists were able to develop standardized, chemically defined culture media that made it far easier to grow cells.

**Types of Animal Cell Culture:**

**i. Primary Culture:**

When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a Primary Culture. There are two basic methods for doing this. First, for Explant Cultures, small pieces of tissue are attached to a glass or treated in a plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant onto the culture vessel surface or substrate where they will begin to divide and grow.[24]

The second, more widely used method, speeds up this process by adding digesting (proteolytic) enzymes, such as *trypsin* or *collagenase*, to the tissue fragments to dissolve the cement holding the cells together. This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide. This method is called Enzymatic Dissociation.

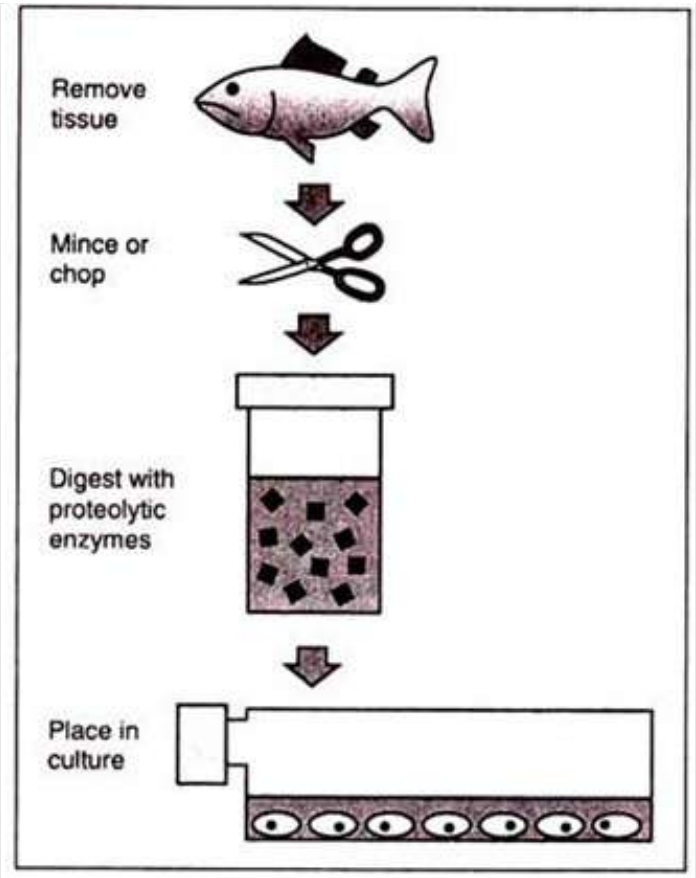



**Fig. 13.2**: The basic steps of Primary cell culture

*Steps of Primary Cell Culture*

**Advantages of Primary Cell Culture:**

The major advantages of primary cultures are the retention of:

**1. The Capacity for Biotransformation:**

In many cases, the metabolism of a primary cell culture has greater similarity to *in vivo* than that seen with subcellular fractions used as an exogenous source for biotransformation.

**2. The Tissue-Specific Functions:**

The second advantage of primary cultures is the retention of tissue specific functions. For example, primary cultures of rat myocardial cells consisting of synchronously beating cells can be prepared. When these cultures were exposed to tricyclic antidepressants that are cardio toxic, beating were observed.

**Limitations of Primary Cell Cultures:**

One limitation of primary cultures is the necessity to isolate cells for each experiment. Procedures to isolate cells require the disruption of the tissue, often with proteolytic enzymes. This may result in the loss or damage of specific membrane receptors, damage to the integrity of the membrane, and loss of cellular products.[25]

**Sub-Culturing:**

When the cells in the primary culture vessel have grown and filled up all of the available culture substrate, they must be Sub-cultured to give them room for continued growth. This is usually done by removing them as gently as possible from the substrate with enzymes. These are similar to the enzymes used in obtaining the primary culture and are used to break the protein bonds attaching the cells to the substrate.

Some cell lines can be harvested by gently scraping the cells off the bottom of the culture vessel. Once released, the cell suspension can then be subdivided and placed into new culture vessels.

Once a surplus of cells is available, they can be treated with suitable cryoprotective agents, such as dimethylsulfoxide (DMSO) or glycerol, carefully frozen and then stored at cryogenic temperatures (below -130°C) until they are needed. The theory and techniques for cryopreserving cells are covered in the Corning Technical Bulletin: General Guide for Cryogenically Storing Animal Cell Cultures.[26]

**4. Characteristics of Cultured Animal Cells:**

**Cell Culture Systems:**

Two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated plastic substrate, called as monolayer culture systems, or floating free in the culture medium called as Suspension Culture Systems.[27]

Monolayer cultures are usually grown in tissue culture treated dishes, T-flasks, roller bottles, Culture Chambers, or multiple well plates, the choice being based on the number of cells needed, the nature of the culture environment, cost and personal preference.

**Suspension Cultures are usually Grown Either:**

1. In magnetically rotated spinner flasks or shaken Erlenmeyer flasks where the cells are kept actively suspended in the medium;
2. In stationary culture vessels such as T-flasks and bottles where, although the cells are not kept agitated, they are unable to attach firmly to the substrate. Many cell lines, especially those derived from normal tissues, are considered to be Anchorage-Dependent, that is, they can only grow when attached to a suitable substrate.

Some cell lines that are no longer considered normal (frequently designated as Transformed Cells) are frequently able to grow either attached to a substrate or floating free in suspension, they are Anchorage-Independent. In addition, some normal cells, such as those found in the blood, do not normally attach to sub-strates and always grow in suspension.[28]

**ii. Types of Cells:**

Cultured cells are usually described based on their morphology (shape and appearance) or their functional characteristics.

**There are three basic morphologies:**

**1. Epithelial:**

Like: cells that are attached to a substrate and appear flattened and polygonal in shape.

**2. Lymphoblast:**

Cells that do not attach normally to a substrate but remain in suspension with a spherical shape.

**3. Fibroblast:**

Cells that are attached to a substrate and appear elongated and bipolar, frequently forming swirls in heavy cultures. It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

Using cell fusion techniques, it is also possible to obtain hybrid cells by fusing cells from two different parents. These may exhibit characteristics of either parent or both parents. This technique was used in 1975 to create cells capable of producing custom tailored monoclonal antibodies.[29]

These hybrid cells (called Hybridomas) are formed by fusing two different but related cells. The first is a spleen-derived lymphocyte that is capable of producing the desired antibody. The second is a rapidly dividing myeloma cell (a type of cancer cell) that has the machinery for making antibodies but is not programmed to produce any antibody.

The resulting hybridomas can produce large quantities of the desired antibody. These antibodies, called Monoclonal Antibodies due to their purity, have many important clinical, diagnostic, and industrial applications with a yearly value of well over a billion dollars.

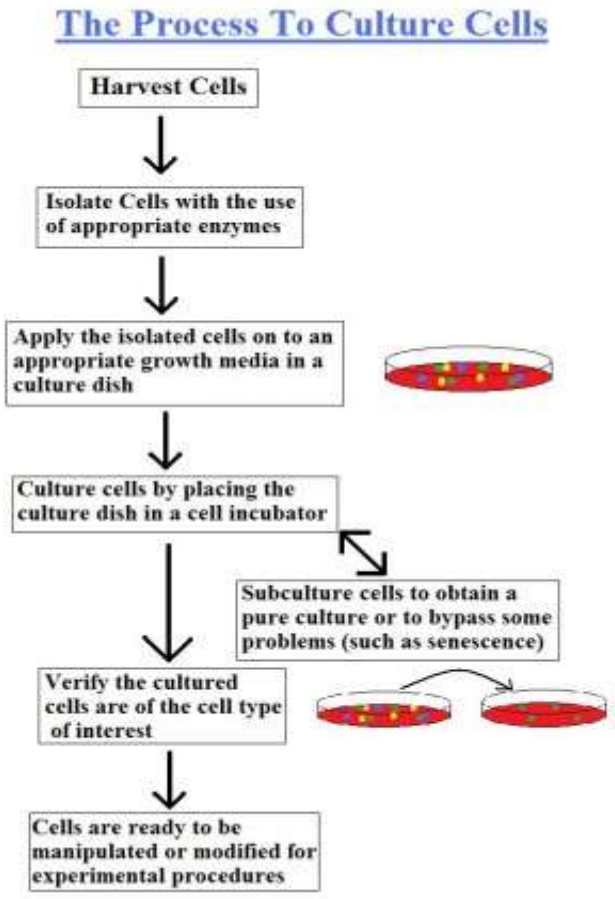
**iii. Functional Characteristics:**

The characteristics of cultured cells result from both their origin (liver, heart, etc.) and how well they adapt to the culture conditions. Biochemical markers can be used to determine if cells are still carrying on specialized functions that they performed in vivo (e.g., liver cells secreting albumin).[30]

Morphological or ultra-structural markers can also be examined (e.g., beating heart cells). Frequently, these characteristics are either lost or changed as a result of being placed in an artificial environment. Some cell lines will eventually stop dividing and show signs of aging.

These lines are called Finite. Other lines which become immortal can continue to divide indefinitely and are called Continuous cell lines. When a "normal" finite cell line becomes immortal, it undergoes a fundamental irreversible change or **"transformation".** This can occur spontaneously or be brought about intentionally using drugs, radiation or viruses.

Transformed Cells are usually easier and faster growing, may often have extra or abnormal chromosomes and frequently can be grown in suspension.Cells that have the normal number of chromosomes are called Diploid cells; those that have other than the normal number are Aneuploid. If the cells form tumours when they are injected into animals, they are considered to be *Neo-plastically* Transformed.[31]




*Process To Culture Cells*

**5. Advantages of Animal Cell Culture:**

a. Controlled physiochemical environment (pH, temperature, osmotic pressure, etc.)
b. Controlled and defined physiological conditions
c. Homogeneity of cell types.
d. Economical, since smaller quantities of reagents are needed than in vivo.
e. Legal, moral and ethical questions of animal experimentation are avoided.

**6. Disadvantages of Animal Cell Culture:**

a. Expertise is needed, so that behaviour of cells in culture can be interpreted and regulated.

b. Ten times more expensive for same quantity of animal tissue, therefore reasons for its use should be compelling.

c. Unstable aneuploid chromosome constitution.

**Applications of Animal Cell Culture:**

**A. Model Systems:**

Cell cultures provide a good model system for studying

a. Basic cell biology and biochemistry.

b. The interactions between disease-causing agents and cells.

c. The effects of drugs on cells.

d. The process and triggers for aging.

f. Nutritional studies.

**B. Toxicity Testing:**

Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important for liver and kidney derived cell cultures.[32]

**C. Cancer Research:**

Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells.

Thus, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.

**D. Virology:**

One of the earliest and major uses of cell culture is the replication of viruses in cell cultures for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.[33]

**E. Cell-Based Manufacturing:**

While cultured cells can be used to produce many important products, They are:

The first is the large-scale production of viruses for use in vaccine production. These include vaccines for polio, rabies, chicken pox, hepatitis B and measles.

The second is the large-scale production of cells that have been genetically engineered to produce proteins that have *medicinal* or commercial value. These include monoclonal antibodies, insulin, hormones, etc. The third is the use of cells as replacement tissues and organs. Artificial skin for use in treating burns and ulcers is the first commercially available product.[34]

However, testing is underway on artificial organs such as pancreas, liver and kidney. A potential supply of replacement cells and tissues may come out of work currently being done with both embryonic and adult stem cells.

These are cells that have the potential to differentiate into a variety of different cell types. It is hoped that learning how to control the development of these cells may offer new treatment for a wide variety of medical conditions.

**F. Genetic Counselling:**

Amniocentesis, a diagnostic technique that enables doctors to remove and culture *fetal cells* from pregnant women, has given doctors an important tool for the early diagnosis of *fetal disorders.* These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.[35]

**G. Genetic Engineering:**

The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists to study the cellular effects of the expression of these genes[36] (new proteins).

These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study. Insect cells are widely used as miniature cells factories to express substantial quantities of proteins that they manufacture after being infected with genetically engineered baculoviruses[37].

**H. Drug Screening and Development:**

Cell-based assays have become increasingly important for the pharmaceutical industry, not just for cytotoxicity testing but also for high throughput screening of compounds that may have potential use as drugs.[38] Originally, these cell culture tests were done in 96 well plates, but increasing use is now being made of 384 and 1536 well plates.

**I. Gene Therapy:**

In modern molecular biology,[39] Gene Therapy is an experimental technique that involves insertion of cloned/altered genes into cells using *r-DNA technology* to replace defective genes causing genetic abnormalities or to prevent potential disorders.

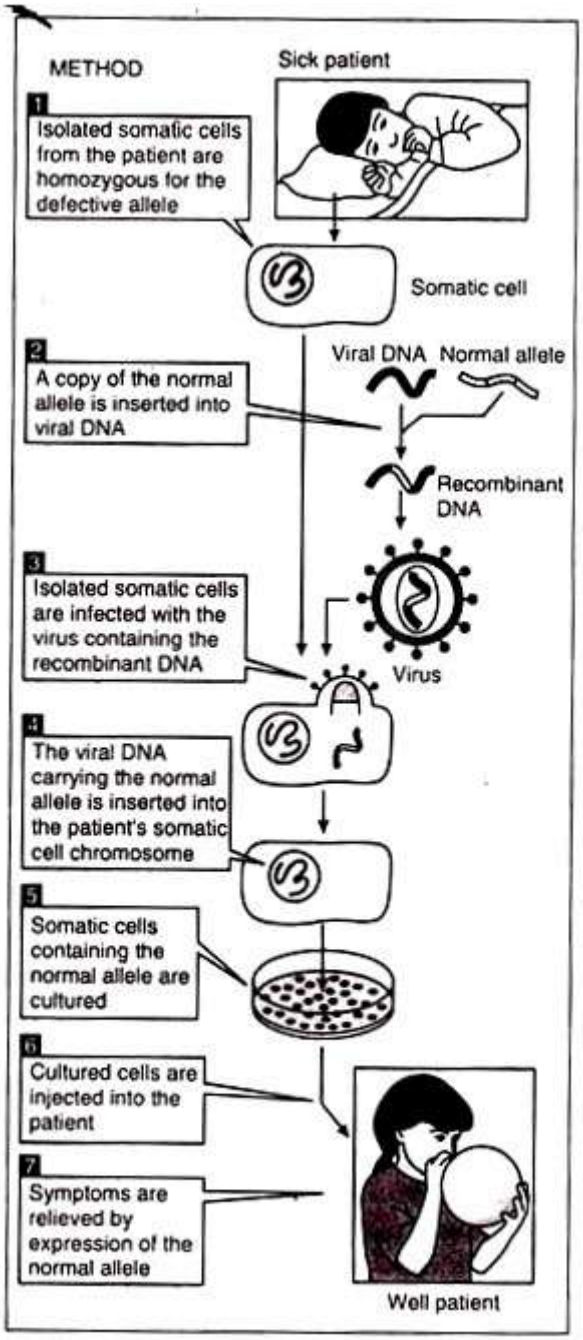



**Fig. 13.5**: The Principle behind Gene Therapy

**Followings are the Uses of Gene Therapy:**

a. Swapping harmful mutant alleles with functional ones by selective reverse mutation.
b. Deactivating improperly functioning mutated gene.
c. Inserting a new gene into the body to help battle a disease.
d. Interchanging non-functional gene with normal gene through homologous recombination[40].

## IV. Result And Discussion

The above data gives a clear information about *"Plant Cell Culture"* and "*Animal Cell Culture Technologies"*and their wide applications.

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