

# It 'callus' and suspension of free cells



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It overcomes the barriers of crops incompatibility in distant crossed (interspecific and intergeneric crosses). It helps in development of transgenic (genetically engineered) plants with resistance to herbicides, biotic and abiotic stresses and improvement in quality. It is a rapid method of crop improvement, e.

g. tetraploid plants can be obtained in one step through protoplast fusion.

**Plant Tissue Culture:** Plant tissue culture refers to growth of living plant tissues in a suitable culture medium (in vitro). In a broad sense, it is referred as cultivation of plant organs, tissues or cells in test tubes on an artificial media; often the techniques of plant cell and tissue culture are also called in vitro techniques. " Culture medium" is a nutrient medium, which contains all essential micro and macronutrients, carbohydrates, vitamins and hormones.

The pH of the nutrient medium should be 5.5. However, the culture medium differs from species to species. In this technique, new plants are regenerated from tissues, cells and organs in test tubes in the nutrient medium. The organ includes any plant organ that has separate identity such as anther, ovule, embryo and bud. The plant part, which is used for regeneration, is called ' explant'. It may be a cell, a protoplast, a tissue or an organ.

A mass of regenerated cells in culture medium is called ' callus' and suspension of free cells of callus in a liquid medium is known as ' suspension culture'. The regeneration capacity or ability of a plant cell to develop into a whole plant is known as ' totipotency'

**Steps in Tissue Culture:**

Tissue culture technique consists of four important steps: 1. Isolation of tissues 2. Regeneration and callus formation in the culture medium. 3. Embryogenesis 4.

Organogenesis 1. Isolation of tissues: Disease free tissues for regeneration can be isolated with the help of sterilized blade from any plant part viz. e. g. stem, apical bud, axillary bud etc. 2.

Regeneration and callus formation: Tissues proliferate on the culture medium and give rise to a mass of cells called ' callus'. The callus is generally of two types: 1. Friable callus 2.

Compact callus The friable callus can be easily manipulated for suspension culture. However, compact callus is not suitable for suspension culture.

**Techniques of Tissue Culture:**

Tissue culture techniques are also referred to as in vitro techniques. In vitro techniques give rise to new plants by regeneration of tissues and organs in the nutrient medium. Various plant parts viz.

meristems, embryo, anther, cells and ovules are used for regeneration.

Depending upon the plant parts used as explants, plant tissue culture techniques are of five main types viz. meristem culture, embryo culture, anther culture, cell culture and ovule culture. (A) Meristem Culture: Meristem culture refers to regeneration of whole plant from tissues of an actively dividing plant part such as stem tip, root tip or axillary bud. Generally, shoot apical meristem is used for regeneration.

This technique is widely used in vegetatively propagated plants such as sugarcane, potato, banana and several fruit trees and timber species. The main applications of meristem culture in crop improvement are: 1. It is used for micro propagation (mass production of clonal progeny through tissue culture) in banana, strawberries, citrus etc. 2.

Virus free plants can be obtained through this technique because meristematic cells are almost free from virus even in the virus infected plants. 3. The exchange of germplasm plantlets obtained by meristem culture is safe, because some material is free from insect and pathogens. This is useful in the exchange of germplasm of asexually propagated plant species. 4. The germplasm can be conserved in the form of meristems of  $-196^{\circ}\text{N}$  for long term storage in the liquid nitrogen i. e.

, meristems are suitable for cryopreservation. (B) Embryo Culture:

Regeneration of a whole plant from an embryo in the culture medium is called 'embryo culture'. Embryos of appropriate stage are removed from the seed and are transferred to the culture medium.

This technique is used when there is disharmony between embryo and endosperm. This technique is used to make distant crosses successful. e.

g. 1. Interspecific hybridization in *Trifolium*, *Lycopersicon* on 2. Intergeneric hybridization in *Hordeum* & *Secale* *Triticum* & *Secale* *Triticum* & *Aegilops* etc.

Main Advantages are — 1. Useful in making interspecific and intergeneric (distant) crosses successfully 2. Useful in obtaining haploids e.

g. barley and wheat. 3. In orchids, seeds are stored food and are unable to propagate. Hence embryo culture is useful 4. Long seed dormancy is noticed in Prunus, Iris and Taxus species.

Embryo culture is useful in breaking the long dormancy and in obtaining seedlings. (C) Anther Culture: Regeneration of whole plant from anther (or) pollen in the culture medium is called anther culture. The optimum stage differs from species to species, e. g. brassica, tobacco, rice, barley, wheat, potato, tomato, triticale etc. Advantages are — 1. Obtaining haploids 2.

Homozygous diploids are obtained simply by doubling chromosomes or obtained spontaneously from anther culture. (a) Mapping population (b) Breeding cycle is shortened (D) Cell Culture: Regeneration of whole plant from callus and suspension cultures in the nutrient medium is known as cell culture. Protoplasts (naked cells or cells without cell wall) are also used for regeneration of whole plants, e. g., wheat, rice, barley, maize, tobacco, fruit and timber trees. Advantages are — 1. Useful in mass clonal propagation of different species. 2.

Protoplast culture is useful in somatic hybridization and overcoming the barriers of cross incompatibility. 3. Useful in the development of transgenic (genetically engineered) plants. 4. Useful/suitable for cryopreservation i. e. preservation in liquid Nitrogen at -196 degree Celsius. (E) Ovule Culture: Regeneration of whole plant from the ovule in the nutrient medium is called ovule culture.

Two types of ovules: Unfertilized > Haploids Fertilized > Diploids This technique is however used to a limited extent.

**Applications in Crop Improvement:**

(A) Generation of Variability Variation is created through invitro techniques. Depending upon the explants, variation induced by tissue cultural techniques is of three types: 1. Gainetoclonal variation: Variation observed among plants, which are regenerated from gametic culture, (anther/ovule culture). 2. Somaclonal variation: The variation observed among plants obtained callus culture of somatic explants such as meristems. 3. Protoclonal variation: The variation observed among plants that are regenerated from callus cultures of protoplast. Main features of the variation are — 1. Variation caused is genetic in origin and hence heritable. 2.

Somaclonal variation occurs both in sexually and asexually propagated species. But, the frequency is very high in a sexually propagated species. 3. Occurs both in oligogenic and polygenic traits. 4. Somaclonal variation is heterozygous in origin (through mutation). 5. All three types of culture variations resulted due to chromosomal changes such as deletion, duplication, inversion and translocations.

Somaclonal variation is useful in isolation of diseases, herbicide tolerant and early maturing variants. (B) Development of Haploids: Made possible through anther and pollen Culture. In China, one variety in wheat (Jighua 1) and in rice (Guan 18) have been developed for commercial cultivation.

**Protoplast Culture:**

Isolated plant protoplasts are cells from which the walls have been removed.

Cell walls can be removed either by mechanical or enzymatic methods.

Isolated protoplasts can be induced to divide in culture to form callus. From the callus whole plants can be regenerated, either through embryogenesis or organogenesis. Isolation of Plant Protoplasts: Plant protoplasts are generally isolated from leaves by enzymatic method. The Need for an Osmoticum: During isolation and culture, plant protoplasts require a suitable osmotic stabiliser. Such a substance is called osmoticum.

In the absence of an osmoticum the protoplasts may burst. Several ionic and nonionic solutes have been tested for adjusting osmotic potential of various solutions used in protoplast isolation and culture. The important osmotica are sorbitol, mannitol, glucose or sucrose. Culture of the Protoplasts: Isolated protoplasts are cultured invitro to regenerate whole plants. The procedure is outlined below.

1. A culture medium (MS medium) with mannitol containing 1.5% agar in a petri dish is used. To this medium the protoplasts are embedded and incubated at 25°C in dim light.
2. Within 48 hours the naked protoplasts form cell walls.

3. Two to seven days after culture, cell division occurs to form callus.
4. The callus is transferred to a medium without mannitol and auxin.
5. Within 3 to 4 weeks embryoids are formed.
6. These embryoids are dissected from the callus and transferred to filter-paper bridge in a culture tube containing liquid medium composed of: MS salts Kinetin- 0.

- 2 mg/l Sucrose - 2.0% w/v
7. After a few weeks plantlets are produced. These are transferred to sterile soil and grown to maturity.

**Applications in Protoplast Culture:**

Protoplast culture has wide applications, some of them are: 1. Cell Biology Studies: Isolated protoplast are ideal materials for the study of cell wall synthesis in plant cells. 2.

Protoplast fusion and Somatic Cell Hybridisation: Two protoplasts from the same source or different sources can fuse to form a hybrid protoplast. Whole plants can be regenerated invitro from this hybrid protoplast. This method is utilised to produce hybrids from two plants which show physical or chemical incompatibility in normal sexual crosses.

When protoplast isolated from two sources are allowed to fuse their cytoplasm mix. The nuclei of the fused protoplasts may fuse nuclei are called heterokaryons or Heterokaryocytes. Thus hybrid protoplasts are formed. These are now selected culture medium to regenerate hybrid plants.

Thus isomatic cell fusion helps to obtain interspecific and intergeneric hybrids which are difficult to obtain by conventional plant breeding methods. This method of obtaining hybrids are called somatic hybrids. Thus production of somatic hybrids is a multistep process: (i) Isolation of protoplasts from two different species (ii) Fusion of protoplasts from two different species (iii) Isolation of fused protoplasts, and (iv) Regeneration of fertile hybrid plants from the fused protoplasts. (v) Somatic hybrids have been produced in several crop plants like potato and tobacco.

3. Protoplast Fusion and Production of Cybrids: When protoplasts from two different species are allowed to fuse, their cytoplasm mix. Sometimes, the



nucleus of one of the protoplasts completely disappears. The cytoplasm of the two parental protoplasts are still hybridized.

This fusion product is called a cybrid or cytoplasmic hybrid. Some genetic factors are carried in the cytoplasm, e. g. factors for male sterility in some plants. Hence formation of cybrids has practical applications in plant breeding.

#### 4. Production of Genetic Variants from Cell/ Protoplast Culture:

Plants regenerated from cell cultures often show genetic variations.

Since, such variation occurs in cells of somatic origin, it is called somaclonal variation. Several kinds of mutations are formed inversion, duplication and deletion. Somaclonal variation in cell culture cannot be directed toward changing a particular kind of mutant. Beneficial genetic variations originating in cell cultures can be utilised in crop breeding for the development of improved varieties of crop plants, using this method genetic variants with traits such as resistance to herbicides, salt tolerance and resistance to toxins such as resistance to herbicides can be evolved. Somaclonal genetic variants have been evolved in several crop plants like wheat and sugarcane.

5. Gene-transfer into Higher plants by Cocultivation of Plant Protoplasts with Agrobacterium: Transgenic plants can be evolved by introducing foreign genes into isolated plant cells/ protoplasts by co-cultivation with Agrobacterium tumefaciens that contains a  $\phi$ -integrate plasmid carrying the foreign gene. The transformed plant cells can be regenerated invitro to evolve transgenic plants.

6. Introduction of Foreign Genes into Isolated Plant cells/Protoplast by Microinjection, Electroporation and Microprojectile Bombardment etc. Foreign

DNA can be introduced into isolated plant cells by microinjection, electroporation and microprojectile bombardment. Liposomes containing foreign genes can be fused with isolated protoplasts. The transformed cells/protoplasts are then selected by growing on suitable media and are then regenerated to produce transgenic plants. Using these methods plants that are resistant to herbicides toxins, pests and pathogens have been evolved. Several transgenic plants that can produce compounds such as vaccines, interferons, plastics and thaumatin have been evolved.

7. Plant cell Cultures and Biosynthesis of Secondary Products: Plant cells can be grown as micro-organisms in culture media and many secondary metabolites have been synthesised from different plant species. The yield of these substances is more when compared with the yield from whole plants.

Table 1: A few secondary products produced by plant cells in culture: Plant

Product	Plant Species
Codeine (alkaloid) - analgesic	Papaver somniferum
Diosgenin (steroid) - antifertility agent	Dioscorea deltoidea
Dogoxin (Cardiac glycoside)	Digitalis lanata
Quinine (alkaloid) - Antimalarial agent	Cinchona ledgeriana
Pyrethrin (insecticide)	Chryanthemum cine rarialfolium
Thaumatococcus danielli	Thaumatococcus danielli
Thaumatococcus danielli	Thaumatococcus danielli
Ginseng - stimulant	Panax ginseng
Jasmine (perfume)	Jasminum sp.

8. Plant Cell Culture in Biotransformation: Plant cell culture can convert certain organic substances added to the cultures into useful products. This process is called biotransformation. Using this method useful substances that have pharmacological properties can be synthesised.

Hardening or Acclimatisation of Plantlets Regenerated Through Culture: The plantlets regenerated through tissue culture methods are transferred to soil

for further growth and development. Different species of plants differ in their capacity to adjust to the new environment. During this period the plantlet has to change from the heterotrophic to the autotrophic state.

Water loss from the regenerated plantlet is high. This is due to the following reasons: 1. The root system formed in the cultured plantlets is inadequate to maintain the plantlets in the soil. 2. The regenerated plantlets have thin leaves with reduced epicuticular wax on their leaves and stems.

3. The stomata do not respond efficiently to stress conditions. Due to these reasons the regenerated plants dehydrate more quickly than the normal plants. Hence the regenerated plantlets must be protected from desiccation and hardened to attain some tolerance to moisture stress. The regenerated plantlets have been grown under aseptic conditions. So, they will be easily attacked by soil pathogens.

Such plants should be protected from soil borne pathogens so that they can grow and develop into healthy plants. Hardening of regenerated plantlets is done by planting them in a sterile soil and covering the plants with a transparent hood. These plantlets are grown in shade for some time and then transferred to the field

#### **Artificial Seeds:**

Somatic embryo (embryoids), shoot buds or any other plant material obtained as a result of invitro culture are covered (encapsulated) with a chemical membrane. Such encapsulated materials behave as seeds. These are called artificial seed's or synthetic seeds.

The artificial covering acts as an artificial seed coat. Such seeds are bead like and can "germinate" and plantlets are also formed. Several substances are used as artificial seed coats. Some of them are agar, agarose, carrageenin, polyacrylamide, introcellulose, ethyl cellulose and sodium alginate.

Sodium alginate is most commonly used. Advantages of Artificial Seeds: 1.

The size of the artificial seeds is smaller when compared to the natural seeds of the plant. 2. Storage and transportation of such seeds is easier. 3. Viability of seeds is 100%.

4. Artificial seeds can be made to germinate uniformly on a suitable substratum. 5. Such seeds do not show dormant. 6. The plant grower can grow the desired plant any time and this is not season dependent. 7.

Large scale production of seeds from any kind of plant part is possible.

Disadvantages: 1. The artificial seeds cannot be stored for longer time and it is temperature dependent. 2. The initial cost for the production of artificial seed is more than that for the natural seeds. 3. Production and germination of artificial seeds require aseptic conditions. Any deviation will affect the quality of the seeds and their subsequent development.

#### **Applications in Plant Tissue Culture:**

Plant tissue culture has applications in agriculture, horticulture, plant breeding and industry. 1. Rescue of hybrid embryos.

2. Culture of immature embryos to propagate orchids. 3. Culture of mature embryos to reduce seed dormancy. 4. Production homozygous lines of recessive genes through anther or pollen culture. 5.

Production of disease free clones through nucellus culture and apical meristem culture. 6. Production of somatic hybrids. 7.

Production cybrids. 8. Production of genetic variants from cell/ protoplast culture.

9. Creation of transgenic plants. 10.

Biosynthesis of secondary products and biotransformation. 11.

Micropropagation.

### **Tissue culture for Germplasm Storage:**

Invaluable plant species are stored to make them available for future breeding programmes. This is called Germplasm storage. Normally it is done by storing the seeds. It has certain limitations: 1. The seeds may lose viability with the passage of time, 2.

The seeds may be damaged by seed borne pathogens, 3. This method cannot be effectively used for vegetatively propagated crops. Tissue culture is used as an alternative method for the storage of germplasm. Culture tissues can be indefinitely stored at very low temperatures in a frozen state. This method is called cryopreservation. When there is a need whole plants can be regenerated from the tissues under cryopreservation.

When there is a need whole plants can be regenerated from the tissues are brought to a state of non-dividing and zero- metabolism. Commonly tissues are stored in liquid nitrogen at  $-196^{\circ}\text{C}$ . Certain substances are added to the culture media before freezing. These protect the tissues against ice damage. Such substances are called cryoprotectants, e. g.

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, Glycerol, Proline, Mannitol, Sucrose, Glucose, Polyethylene glycol.

Cryopreserved tissues can be recultured to produce, whole plants or they can be sub-cultured. The viability of cryopreserved tissues depends upon the plant species.