

# APPLICATION OF PLANT TISSUE CULTURE IN BIOTECHNOLOGY

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## INTRODUCTION

Plant tissue culture is defined as "The culture of plants, seeds, & embryos, organs, explants, tissues, cells and protoplasts of higher plants, in nutrient media under sterile conditions"

These techniques are characterised by

- The exclusion of all micro organisms for growth.
- Possible breakdown of the normal pattern of development.
- Plant improvement by manipulation of tissues.

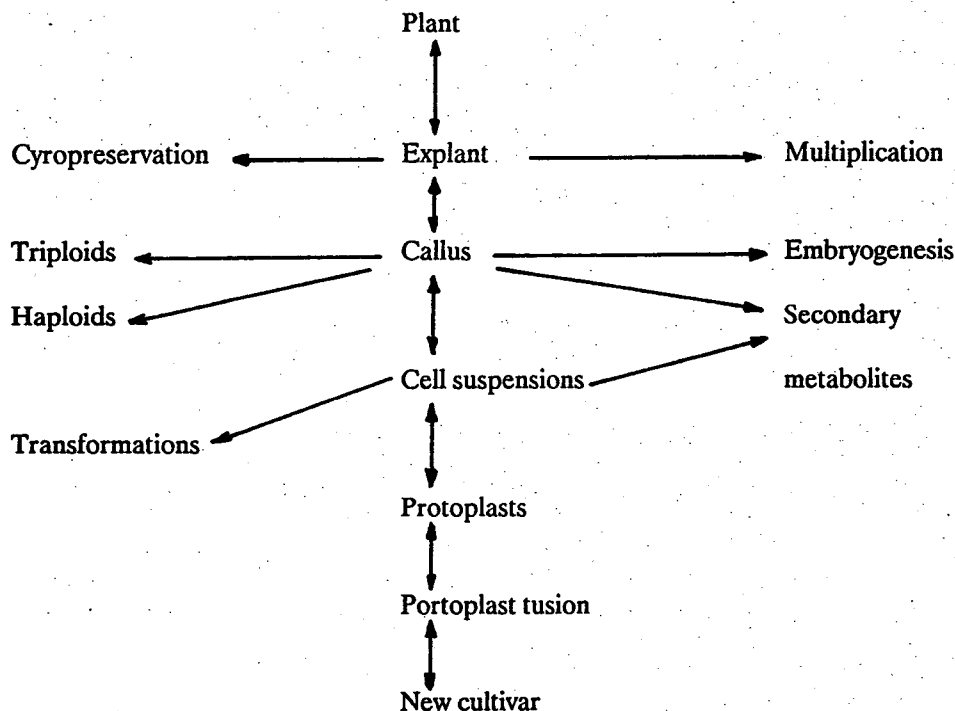
Biotechnology is broadly defined as,

'Any techniques that uses living organisms or parts of organisms to make or modify products to improve plants or animals or to develop micro-organisms for specific uses'.

Traditional biotechnology has been concerned with utilization of micro-organisms in fermentations to produce foods such as tempe, soybean and fish sauces, beer, wine and pharmaceutical products.

Modern biotechnology is based on the use of more recently available technologies, such as new methods of cell and tissue culture, genetic engineering (transformation systems) and new diagnostic techniques. These recent developments have opened up exciting possibilities to increase the efficiency of agricultural production by improving plants, controlling their pests and enhancing their nutrition.

Tissue culture is one of the basic skills required for biotechnology. It is widely used in many developing countries and is often a way to commence building its capacity in more sophisticated methods of biotechnology. It is also a biotechnology which has a more immediate impact on crop production in developing countries. Various manipulation of tissue culture can lead to the improvement of plants.



**The various manipulations that are possible in plant tissue culture.**

Type of plant tissuecultures	Uses
1. Embryo culture	Shortening breeding cycle Overcoming self incompability. Overcoming seed dormancy.
2. Meristem culture	Elimination of pathogens. Mass cloning of plants. Germplasm collection, cryo-preservations.
3. Callus culture Cell culture	Mass cloning of plants. Generation of genetic variants. Elimination of pathogens. Source of protoplasts. Production of secondary metabolites.
4. Anther culture	Production of homozygotes. Induction of mutations.
5. Protoplast culture	Somatic hybridization. Transformation.

Table 1- The various types of plant cell and tissue culture and their uses for plant improvement.

## TYPES OF PLANT TISSUE CULTURE

### Embryo culture

This is the sterile isolation and growth of mature or immature zygotic embryos in vitro to obtain a viable plant. This technique has been used for a number of purposes such as,

- Embryo rescue after intergeneric crosses. eg. Oat x wild oat, Peanut x wild Arachis, Soybean x wild Glycine
- Breaking the dormancy of hard to germinate seeds
- Shortening the breeding cycle.
- Production of a viable plant after enforced self-fertilization.

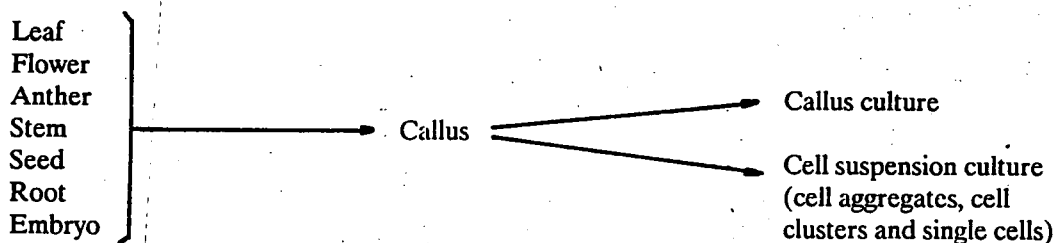
### Meristem culture / shoot tip culture

Meristem culture is the sterile isolation and growth of shoot tips or meristems in vitro in order to obtain clones (micropropagation), virus-free plants or to store germplasm (cryo-preservation).

The most commonly used technique is the rapid production of clones. eg. Bannana, Potato, Eucalyptus, Strawberry, Orchids, etc.

### Callus and cell culture:

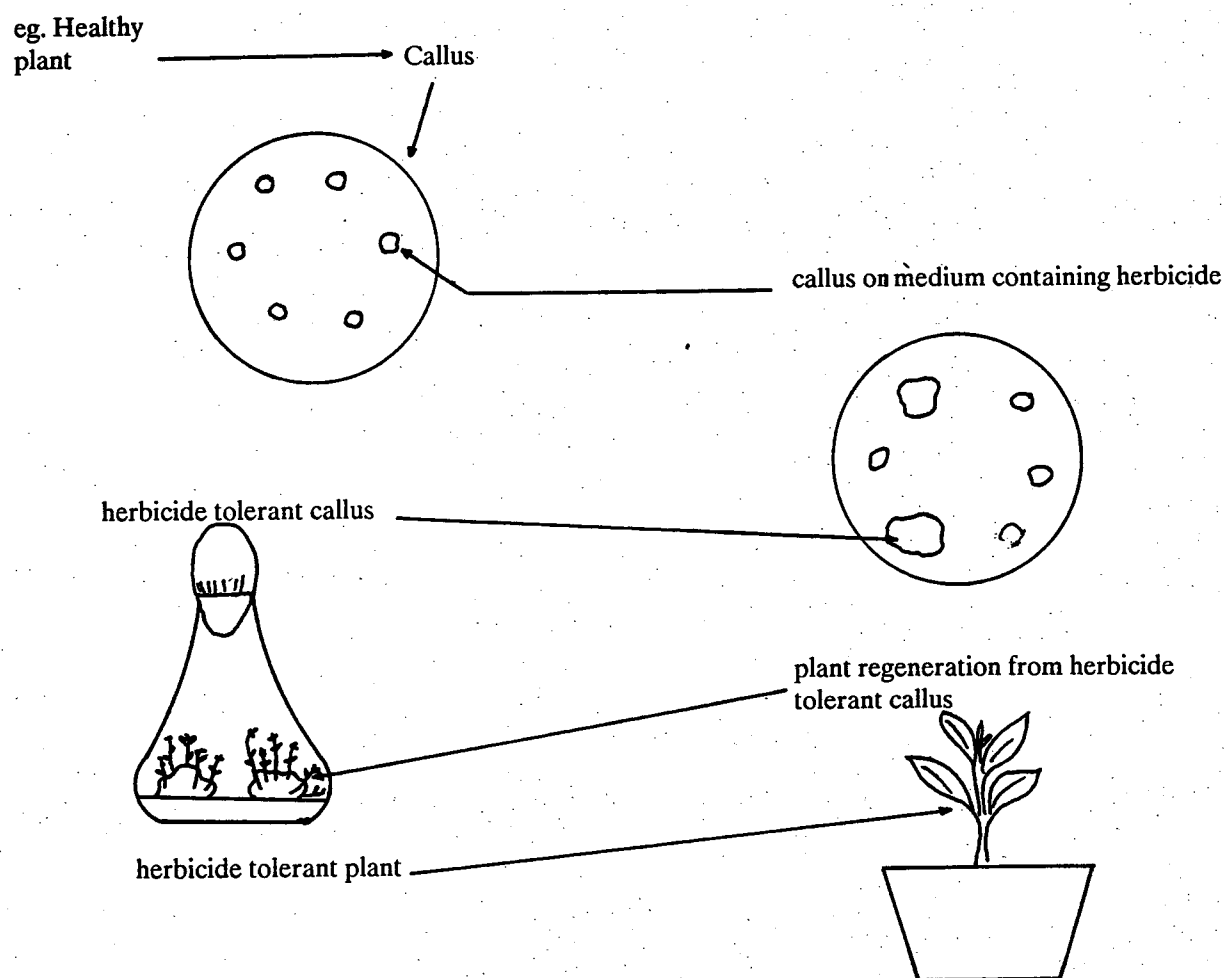
Callus is a mass of unorganized cells.



Callus culture is the sterile in vitro growth of callus with the goal of obtaining improved plants or secondary plant products.

Callus is formed on an explant (part of leaf, stem etc) by culturing in a medium supplemented with a high auxin:cytokinin ratio. eg. of auxins-2,4 - dichloroacetic acid, alpha naphthaleneacetic acid, cytokinins - kinetin, 6 - benzylaminopurine.

The callus formed can be proliferated by subculturing to increase the quantity of callus. On subculture to a medium with low auxin: cytokinin ratio, the unorganised cells begin to get organised and differentiate morphogenetically and produce shoots, plantlets or somatic embryoids. Therefore it can be used to propagate plants. But very often the plants produced show variation among themselves. This is called somaclonal variation. Although this is a disadvantage in mass cloning of plants, somaclonal variation has its advantages. The cell lines can be screened to get desirable characters such as tolerance to drought, salt, herbicides, pathogens or viruses.



Callus can be used to initiate cell suspension cultures by transfer to liquid medium in a rotary shaker. The callus breaks up to form cell aggregates, clusters and single cells.

Cell suspension cultures are used in,

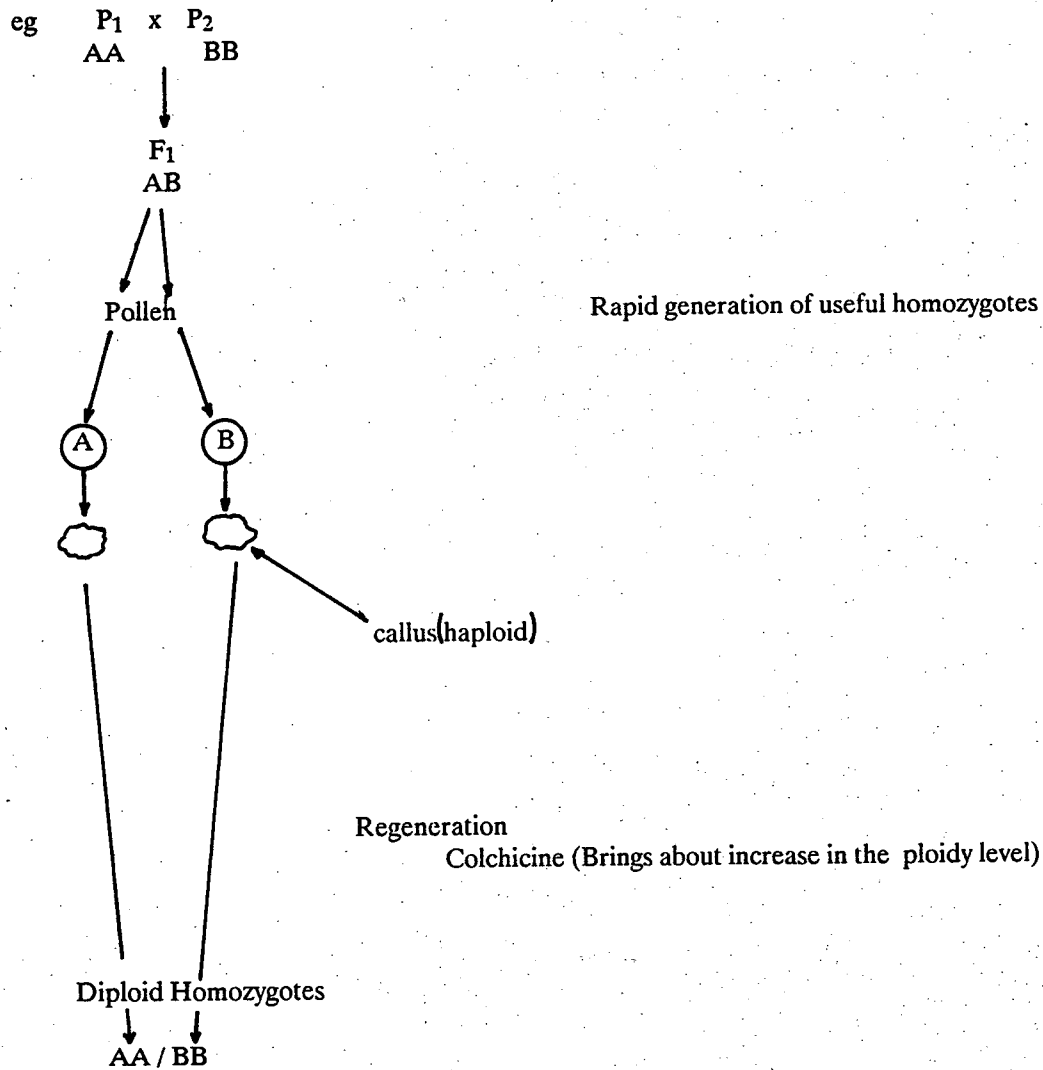
- Proliferation of callus
- Differentiation of somatic embryoids
- Production of secondary metabolites

Callus cultures are also used in the transformation of plants using vectors such as *Agrobacterium tumifaciens*

## Anther Culture:

Anther culture is the sterile isolation of anthers and development of haploid callus cultures from the pollen in vitro. The technique is used for the rapid production of homozygotes.

Normal Breeding methods to get diploid homozygotes takes about 8 generations. Therefore anther culture is a way of shortning the breeding cycle.

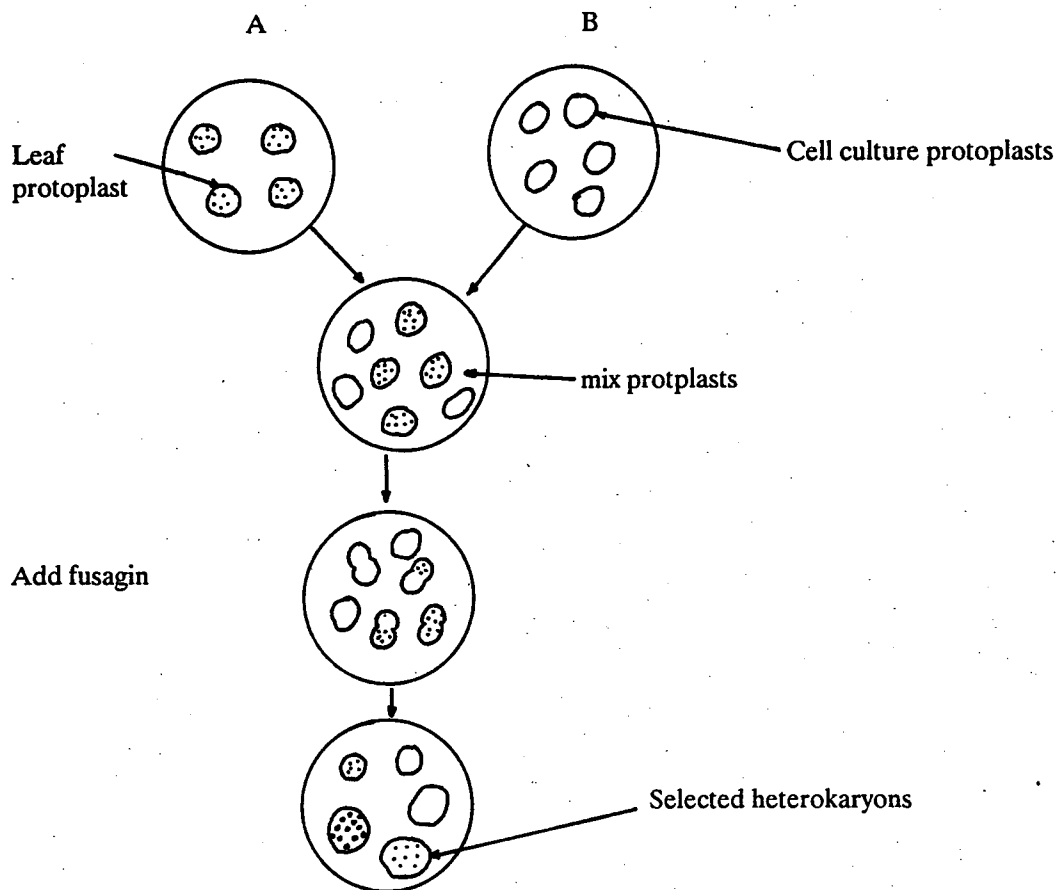


## Protoplast culture :

Protoplast culture is the sterile isolation of cell protoplasts, with the goal of genetically modifying the cell.

Protoplast culture is used in cell modification techniques such as protoplast fusion and direct DNA injection (by electroporation, micro-injection and microprojectile bombardment).

The most commonly used technique is protoplast fusion. Somatic cell protoplasts of two widely different species can be fused to combine desirable traits such as disease resistance.



Protoplast fusion of leaf protoplast of species A with somatic cell protoplasts of species B.

### **AIDS TO STUDY TISSUE CULTURE:**

#### **Literature Survey**

Invaluable help for *in vitro* culture of higher plants can be obtained from handbooks, congress and symposium abstracts and reports and articles in journals.

#### **Tissue culture laboratory**

A specially equipped and maintained laboratory is needed.

Tissue culture involves four main steps.

- Preparation of culture media
- Sterilization of culture media, and vessels.
- Surface sterilization of explants and their inoculation in culture media.
- Incubation of cultured explants.

It is necessary to carry out these steps in separate but adjoining rooms.

### **EQUIPMENT IN A TISSUE CULTURE LABORATORY:-**

#### **MEDIA PREPERATION ROOM:-**

- Balances - Analytical balance
- Top loading balance
- Hot plates
- Strirrers
- pH meter

- Racks and cupboards for storage of chemicals and glassware
- Refrigerators and freezers for storage of chemicals and stock solutions.
- Water distillation and deionisation apparatus.

#### **STERILIZING ROOM:-**

Autoclaves  
Sterilizing ovens

#### **INOCULATING ROOM:-**

Laminar flow hood  
Trolleys  
Side tables  
Stereo microscope

#### **INCUBATION ROOM:-**

Incubators, racks with fluorescence lights shakers, microscopes.

A washing room is also needed for washing used glassware. Depending on availability of space a part of the sterilizing or media preparation room can be used for this purpose.

Some tissue culture laboratories have a separate room for microscopes. Here again depending on availability of space microscopes can even be placed in the inoculating or incubating rooms.

#### **Tissue Culture Media:**

Culture media must contain all nutritional components necessary for growth of a normal healthy plant. These being,

1. Water:- for growth and development
2. Carbohydrates:- Tissues are not completely autotrophic. therefore a source of carbohydrates Such as sucrose, is added to the medium.
3. Mineral nutrients:- These are required for growth and development. All the macro and micro nutrient are incorporated in the form of inorganic salts.
4. Plant growth regulators:- These bring about cell division and growth and differentiation. Different types of regulators are incorporated in various concentration to get the desired type of growth. (See next page for details)
5. Other organic substances:-  
Amino acids  
Vitamins  
Yeast extract  
Coconut water  
Casein hydrolysate
6. Agar - It is added to modify the physical properties of the medium. It is an inert substance which gives a semi solid consistency to the medium.
7. Activated charcoal - Adsorbs toxins produced by tissues
8. Acid/alkali - The pH of the medium is important for healthy growth. It is adjusted to between 5.6 - 5.8 in most media.

#### 4. Plant Growth Regulators:

Certain compounds occur naturally within plant tissues which have a regulatory rather than a nutritional role in growth and development. These compounds are active in very low concentrations and are termed plant hormones (or plant growth substances). Synthetic substances with similar physiological activities are termed plant growth regulators. When these substances are added to plant tissue culture media they are called plant growth regulators.

There are several classes of these plant growth substances.

- Auxins
- Cytokinins
- Gibberellins
- Ethylene
- Abscisins (or dormins)

The first two classes are the most important for regulating growth and morphogenesis in plant tissue cultures.

Plant cells have the ability to synthesize growth substances. They are synthesized in actively dividing tissues. Growth and differentiation in tissue cultures are regulated, by the interaction and balance between growth regulators produced by the culture cells and those supplied in the medium.

The basic mechanisms in regulation in cells is not fully understood. Further the type of plant hormones in a cultured tissue or organ can vary according to the season of the year when the tissue is taken and part of the plant from where the tissue is taken. They vary from species to species and among varieties as well.

Therefore the choice of a compound to be incorporated in the medium and its concentration required will depend on,

The type of growth or development required.

- The natural level of growth regulators found within the tissue at the time it was excised.
- The capacity of these cells to synthesize these substances.
- The interaction, if any, between the natural substances present in the tissue and those applied to the medium.

#### Auxins:

Control cell growth and cell elongation. The most commonly detected natural auxin is Indole 3-acetic acid (IAA). Synthetic auxins are used in tissue culture. IAA can be synthesized.

eg. of Synthetic auxins.

- 2,4-dichlorophenoxyacetic acid (2,4-D)
- alpha naphthaleneacetic acid (NAA)
- Indole butyric acid (IBA)

Effects of auxins in tissue culture:

- Often brings about callus induction.
- Root induction
- Growth of meristem and shoot tips
- Somatic embryogenesis

#### Cytokinins:

These are necessary for cell division. Naturally occurring cytokinins are Zeatin, 2-iP, IPA.

eg. of Synthetic cytokinins

- Kinetin
- BAP (Benzylamino purine)

Effects in tissue culture:

- Stimulation of cell division.
- Adventitious shoot formation
- Axillary shoot proliferation
- Inhibition of root formation.

**Composition of MS Medium (Murashige and Skoog 1962)**

Compound	mM l <sup>-1</sup>	mg l <sup>-1</sup>
<b>Macro nutrients</b>		
NH <sub>4</sub> NO <sub>3</sub>	20.6	1650
KNO <sub>3</sub>	18.8	1900
CaCl <sub>2</sub> · 2H <sub>2</sub> O	3.0	440
MgSO <sub>4</sub> · 7H <sub>2</sub> O	1.5	370
KH <sub>2</sub> PO <sub>4</sub>	1.2	170
<b>Micro nutrients</b>		
	<b>μM l<sup>-1</sup></b>	
MnSO <sub>4</sub> · 4H <sub>2</sub> O	100	22.3
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	30	8.6
H <sub>3</sub> BO <sub>3</sub>	100	6.2
KI	5	0.83
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	1.0	0.25
CuSO <sub>4</sub> · 5H <sub>2</sub> O	0.1	0.025
CoCl <sub>2</sub> · 6H <sub>2</sub> O	0.1	0.025
Na <sub>2</sub> EDTA	100	37.3
Fe SO <sub>4</sub> · 7H <sub>2</sub> O	100	27.3
<b>Organic compounds</b>		
Thiamine. HCL	0.3	0.1
Nicotinic Acid	4.0	0.5
Pyridoxine	2.4	0.5
Glycine	26.6	2.0
Surcose	876 mM.	30,000.