APPLICATIONS OF PLANT TISSUE CULTURE IN BIOTECHNOLOGY

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INTRODUCTION:

Plant development consists of the overlapping activities of growth such as cell division and differentiation. Even a small plant will have millions of cells in it, all containing the same genetic information. All these cells can be traced back to a single cell, the fertilized egg cell. As these cells divide and become parts of tissues and organs they undergo diversification in structure and function. This type of organization enables the individual units to act together for a purpose. A basic problem in biology is to explain the mechanism by which cells with the same genotypic constancy achieved phenotypic diversity of development. This is explained as due to selective gene activity, so that different genes are active in different cells. Further, the remarkable display in totipotency in plant cells enables a differentiated cell to undergo reprogramming of its gene expression, so as to regenerate a whole plant either naturally or under experimental conditions. It is not known how or why certain cells undergo reprogramming of their gene expression in this manner.

It was the realization of the tremendous potential of cellular totipotency that paved the way to tissue culture. This character of totipotency has been exploited in the clonal propagation of plants, production of pathogen free plants, development of homozygous diploid plants through haploid induction, and the use of protoplasts as tools for genetic engineering.

Plant tissue culture is defined as the culture of plants, seeds, embryos, organs, tissues, cells and protoplasts of higher plants in nutrient media under sterile conditions.

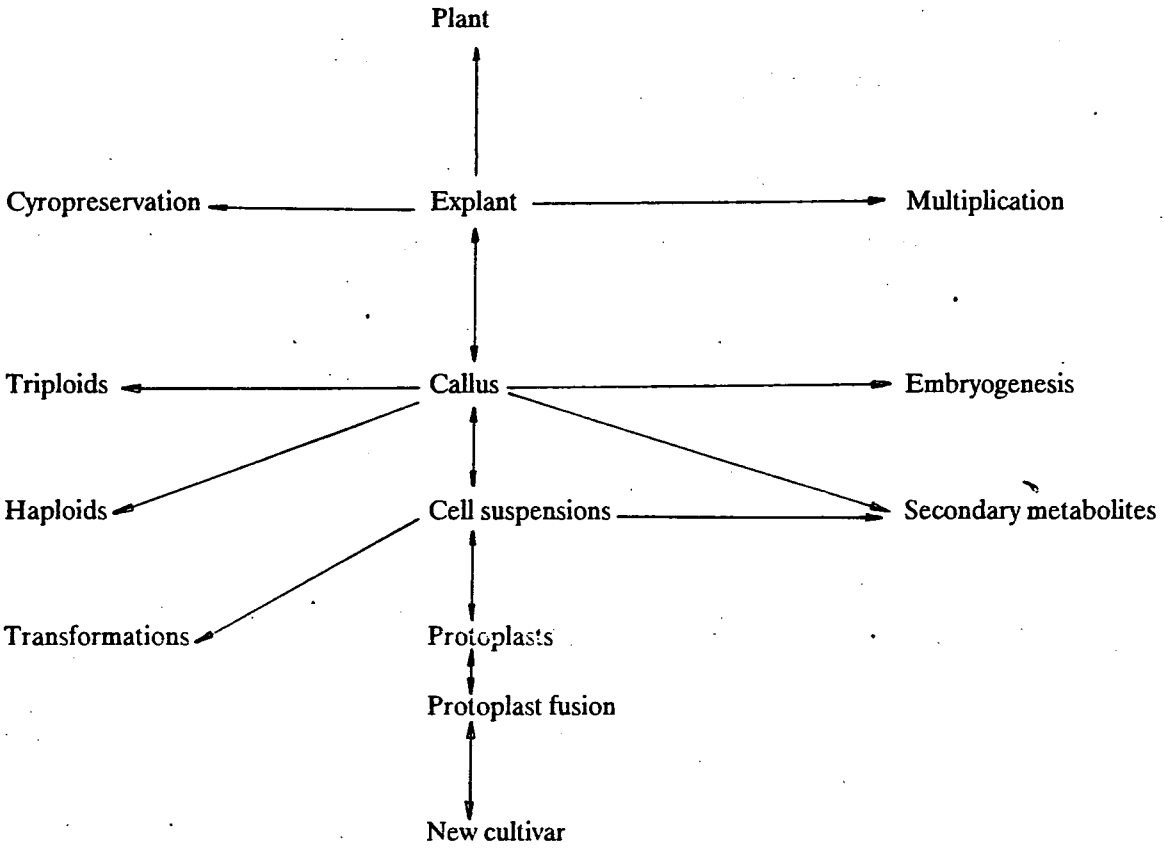
These techniques are characterized by,

- The exclusion of all micro organisms in cultures.
- Possible breakdown of the normal pattern of growth.
- Plant improvement by manipulations of tissues.

Biotechnology is the use of any living organism or part of an organism to make or modify products, improve plants or animals or develop micro-organisms for specific uses.

The utilization of micro-organisms in fermentations to produce foods such as tempe, soybean and fish sauces, beer , wine and pharmaceutical products are examples of traditional biotechnology. Modern biotechnology is based on the use of more recently available technologies such as new methods of cell and tissue culture, genetic engineering (transformations) 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 technology which has a more immediate impact on crop production in developing countries. Various manipulations of tissue culture can lead to the improvement of plants.



The various manipulations that are possible in plant tissue culture.

TYPES OF PLANT TISSUE CULTURE

Type of plant tissue cultures	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. Transformations

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

Embryo culture:

This is the sterile isolation and growth or 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. e.g. 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.

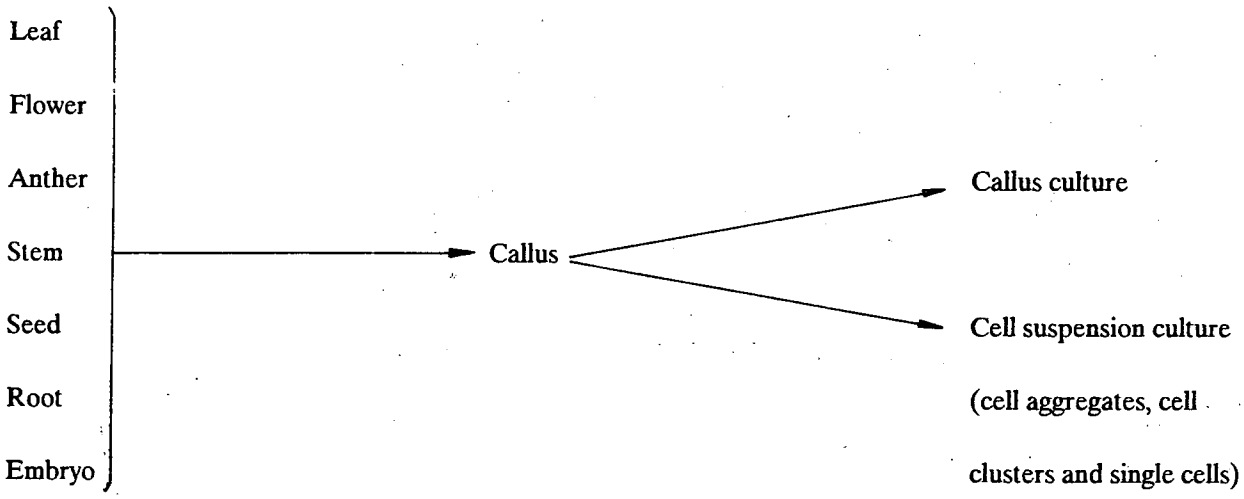
Meristem culture/shoot tip culture :

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. Banana, Potato, Eucalyptus, Strawberry, Orchids, etc.

Callus and cell culture:

Callus is a mass of unorganized cells.

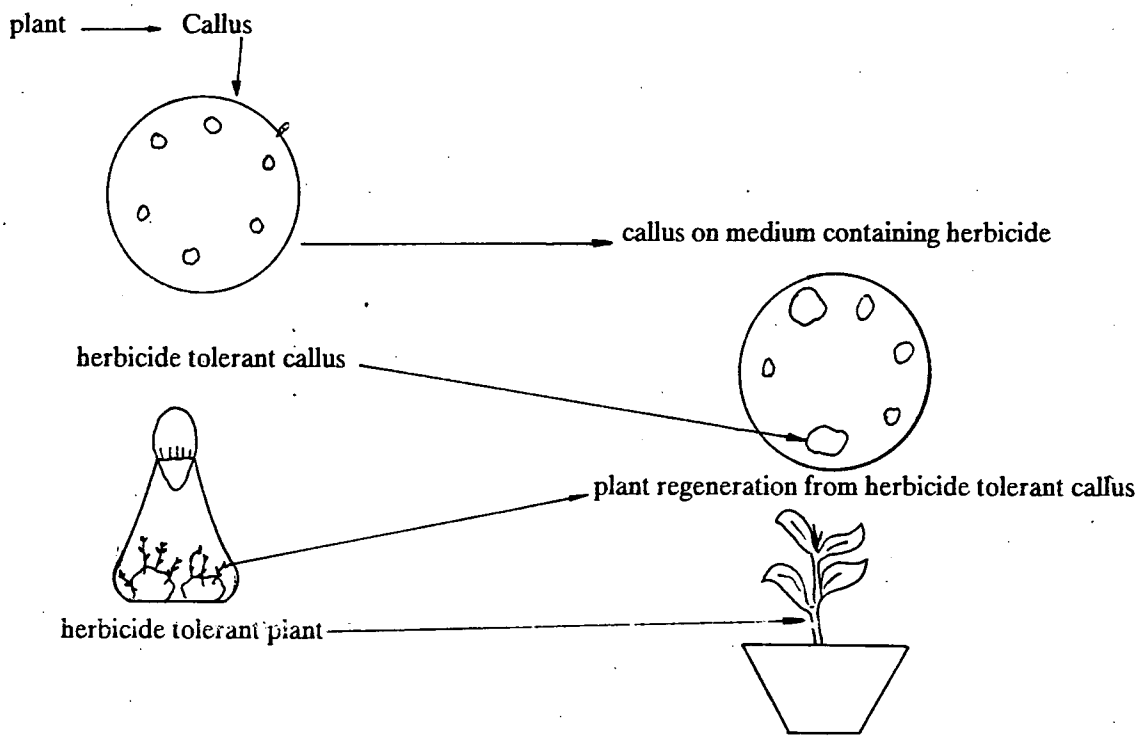


Callus culture is the sterile *in vitro* growth of callus with the goal of obtaining improved plants or secondary plant metabolites.

Callus is formed on an explant (part of leaf,stem etc) by culturing in a medium supplemented with a high auxin:cytokinin ratio. Examples of auxins are 2,4-dichloroacetic acid, alpha nephthaleneacetic acid Examples of cytokinins are 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 ration, the unorganized cells begin to get organized and differentiate morphogenetically and produce shoots, plantlets or somatic embryoids. Therefore this method 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.

eg. Healthy



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. This is called a cell suspension.

Cell suspension cultures are used in,

- *Proliferation of callus
- *Differentiation of callus or cells into somatic embryoids
- *Production of secondary metabolites

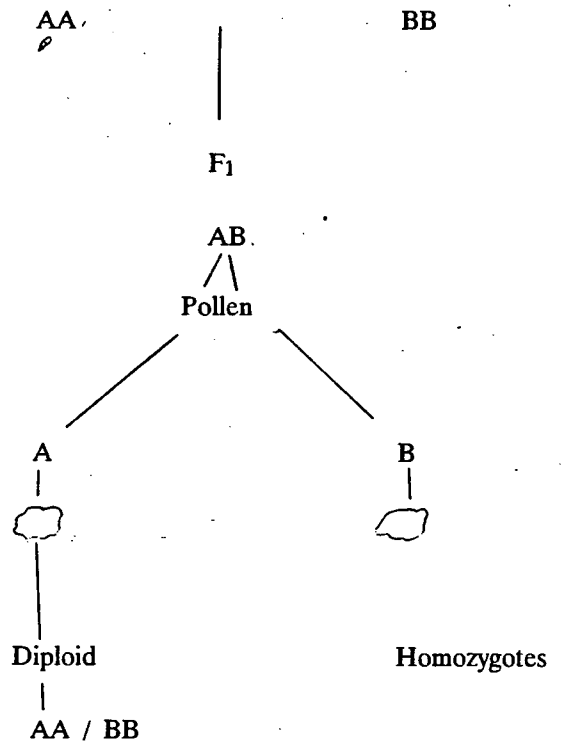
Callus cultures are also used in the transformation of plants using vectors such as *Agrobacterium tumifaciens*

Anther culture:

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

Normal Breeding methods to get diploid homozygotes take about 8 generations. Anther culture is a way of shortening the breeding cycle. Anther culture at the F_1 stage can give rise to homozygons lines in a short period.

eg $P_1 \times P_2$



Rapid generation of useful homozygotes

haploid callus

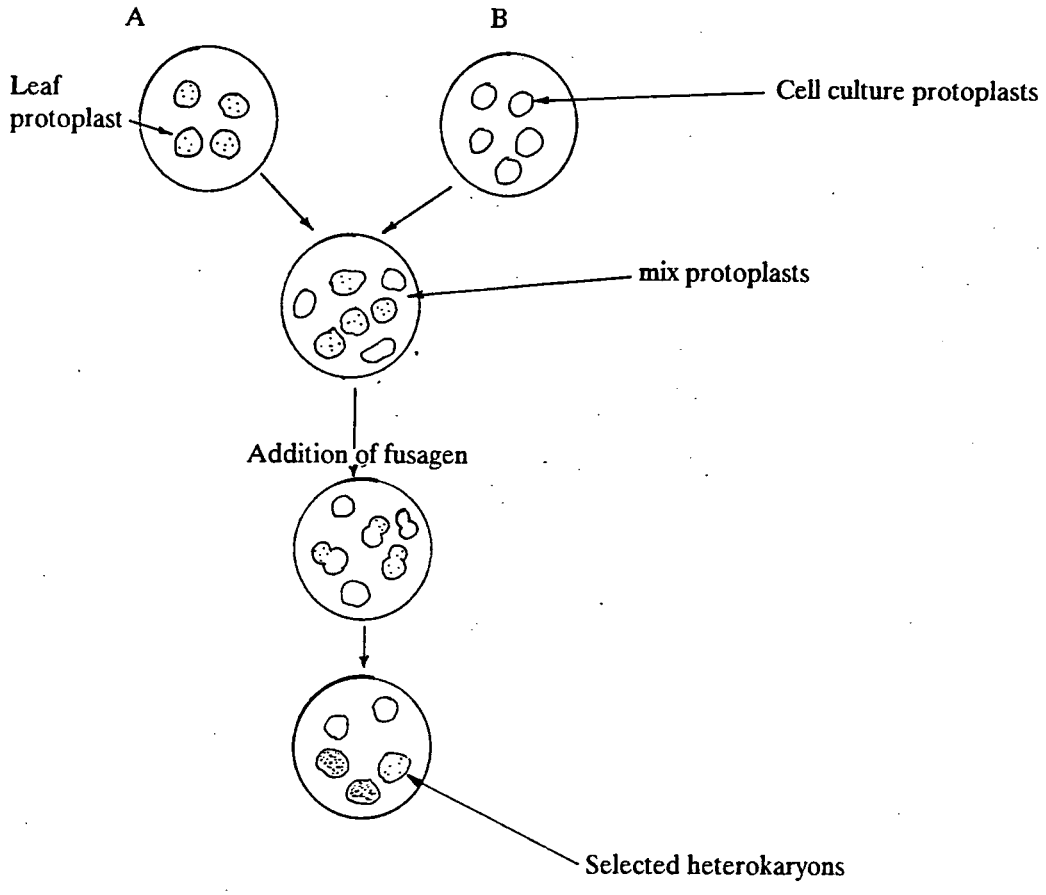
Regeneration (Use of Colchicine brings about increase in the ploidy level

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 used 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 preparation room:-

- Balances-Analytical balance, Top loading balance
- Hot plates
- Stirrers
- pH meter
- Racks and cupboards for storage of chemicals and glassware
- Refrigerators and freezes for storage of chemicals and stock solutions.
- Water distillation and deionization 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 places 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 is added to the medium. The most commonly used is sucrose, Glucose, fructose maltose etc are also used at times.
3. Mineral nutrients: These are required for growth and development. All the elements required in macro and micro quantities are incorporated in the medium in the form of inorganic salts.
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 or plant growth regulators). Synthetic substances with similar physiological activities are also present. These are incorporated in media to bring about desired type of growth.

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 cultured cells and those supplied in the medium.

The basic mechanism in regulation in cells is not fully understood. Further, the type of plant hormones in a cultured tissue or organ can very 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 also be synthesized.

Some examples of Synthetic auxins.

- 2,4-dichlorophenoxyacetic acid (24D)
- 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.

Some examples of Synthetic cytokinins

- Kinetin
- 6-Benzylamino purine (BAP)

Effects of cytokinins in tissue cultures,

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

5. Other organic substances incorporated in tissue culture media:-
   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 innert 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. Generally a pH between 5.6-5.8 brings about optimum growth.

Composition of MS Medium (Murashige and Skoog 1962)

Compoundm	Ml^{-1}	mgl^{-1}
Macro nutrients		
$NH_4 NO_3$	20.6	1650
KNO_3	18.8	1900
$CaCl_2. 2H_2O$	3.0	440
$MgSO_4. 7H_2$	O1.5	370
$KH_2 PO_4$	1.2	170
Micro nutrients	Ml^{-1}	
$MnSO_4. 4H_2O$	100	22.3
$2nSO_4.7H_2O$	30	8.6
$H_3 BO_3$	100	6.2
KI	5	0.83
$Na_2MoO_4. 2H_2O$	1.0	0.25
$CuSO_4. 5H_2O$	0.1	0.025
$CoCL_2. 6H_2O$	0.1	0.025
Na_2 EDTA	100	37.3
Fe $So_4. 7H_2O$	100	27.3
Organic compound	**mM.**	
Thiamine. HCL	0.3	0.1
Nicotinic Acid	4.0	0.5
Pyridoxine	2.4	0.5
Glycine	26.6	2.0
Sucrose	876	30,000.