PLANT TISSUE CULTURE

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Introduction

Plant tissue culture is the culture of a cell, tissue, organ (part of a plant) or a whole plant in an artificial culture medium under aseptic conditions. The cells of the cultured plant or plant part can be made to divide and differentiate as required. This is due to the remarkable feature of plasticity of plant cells. A plant cell which is specialized to carry out a function or a group of functions can be made to revert back to an embryonic state (de-differentiation) of cell division to give rise to undifferentiated cells, which can be again made to differentiate (re-differentiate) into various types of specialized cells or even entire plants. This remarkable feature in plants cells, termed totipotency is exploited to the maximum in plant tissue culture. Totipotency is the most fundamental feature of plant cells that distinguishes them from animal cells. (An animal cell can only be induced to divide to from cells similar to itself and cannot be made to redifferentiate into any other type of cell). Techniques of plant tissue culture have been developed where tens of thousand entire plants can be produced from a small group of cells separated from a selected mother plant. This technique known as micropropagation, is only one of the many uses of tissue culture techniques.

Tissue culture is also a tool applied in modern biotechnology. It is now possible to manipulate the genetic material of a plant by the direct introduction of genes from other plants, animals or micro organisms or remove genes, to investigate their behaviour or to produce a transgenic plant.

Applications 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 many uses.

***Embryo rescue:**

In enforced self fertilization in cross pollinated species or in wide crosses, the development of the zygote into an embryo may not take place within the seed due to various barriers that prevent its development. Early rescue of the embryo and its growth in an artificial medium could help in developing a viable plant.

***Breaking the dormancy of seeds:**

The embryo may be isolated and germinated *in vitro* to overcome dormancy.

***Shortening the breeding cycle:**

A breeding programme requires the raising of a number of generations after crossing. In crops that produce seeds with a long dormant period, the life cycle can be shortened by *in vitro* germination of isolated embryos. eg. in certain crops the seeds to require to go through a long winter period before they will germinate.

***Germplasm exchange:**

Seeds of many tree species rapidly lose viability on dehydration and are recalcitrant to storage under methods that are nomally practiced for most seeds. Seeds of such species cannot be shipped over long distances as they would lose their viability on arrival at the destination. Shipping of *in vitro* cultured embryos is a way of overcoming this. It also has the advantage of having a lower bulk for shipment and of quarantine guarantee due to the absence of microorganisms.

Meristem/shoot tip culture:

Meristem or shoot tip culture is the sterile isolation and growth of the apical meristem or the shoot tip *in vitro*. It has many applications.

*** Micropropagation:**

This method is most commonly used in microprpagation for the rapid production of propagules. The meristem or the shoot tip can be induced to proliferate axillary shoots rapidly. These shoots are rooted to form entire plants eg (Banana, Potato, Eucalyptus, Strawberry, Orchids, etc).

*** Production of virus free plants:**

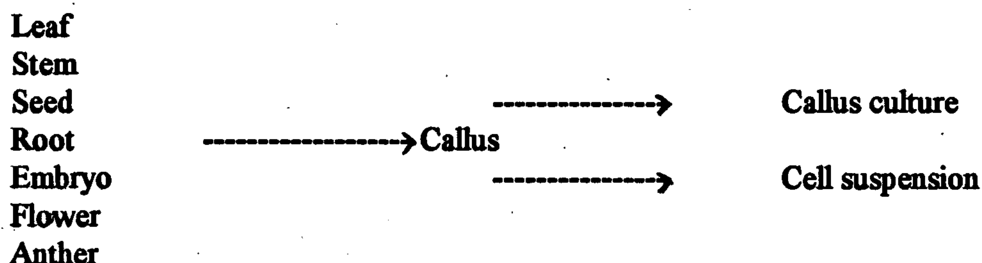
Meristem culture is used in producing virus free plants.This is due to the absence of virus particles in the rapidly dividing cells in the meristem of infected plants. Sometimes it is necessary to give a heat treatment to destroy the virus particles before meristem culture.

*** Germplasm storage:**

Meristem or shoot tip culture is also used as a method of germplasm storage in plants that do not produce seeds or produce seeds that are recalcitrant. They can be stored at low temperatures in liquid nitrogen after pretreatments to minimise cell damage which can take place during the process of freezing.

Callus and cell culture:

Callus is a mass of rapidly dividing cells that are initially not organised. Callus can be induced from various plant parts such as those of,



It is easier to induce callus from cells that are dividing or relatively undifferentiated in the mother plant. The callus may vary in appearance or texture. Some types of callii can be used to form suspensions in liquid media . The suspended cells may separate into single cells and smalll aggregates that undergo cell division and proliferate. Once a callus or a suspension culture is initiated , the cells are allowed to proliferate further to increase the quantity. Next they are induced to differentiate. This can result in the formation of shoots or roots or somatic embryoids. Complete plants can be developed from shoots or from somatic embryoids.

***Artificial seeds:**

Somatic embryoids can be encapsulated in a suitable polymer such as calcium alginate to form an artificial seed. Somatic embryos in encapsulated form has been produced in various species Alfalfa and *Dorcas carota.*

Somaclonal variations:

Both callus and cell suspensions may be induced to undergo differentiation to finally produce complete plants. Very often the regenerated plants show variations. This is called somaclonal variation. This is caused by changes in the chromosomes or genes due to the rapid rate of cell division in callus cells. Although somaclonal variation is a disadvantage in mass cloning of plants, it has certain advantages. Mutants with desirable characters may be selected from the clones. This can be used as a method of producing new genotypes that differ in only a few traits from the stable lines. However deleterious changes are more common than favuorable ones.

The cell lines can also be screened to get desirable characters such as tolerance to drought, salt, herbicides, pathogens or viruses. Plantlets with the desired character will differentiate from the selected cell lines.

*** Transformations:**

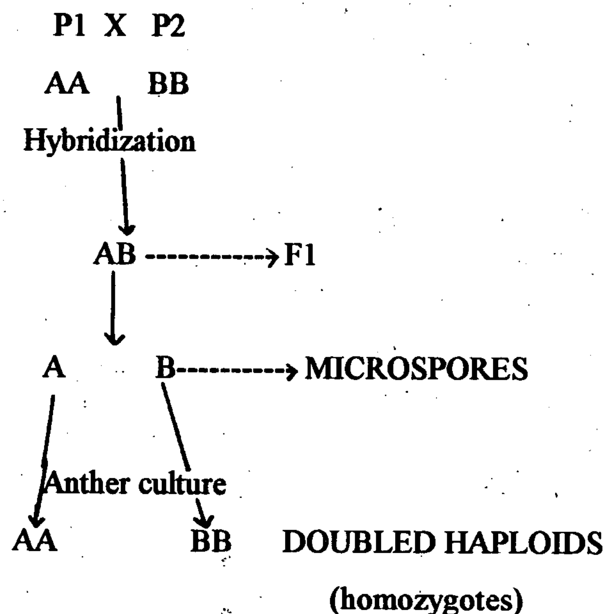
Callus and cell suspensions can be used in the formation of transgenic plants. ie, to develop a plant into which a foreign gene has been introduced. The gene is first introduced into a vector such as *Agrobacterium tumifaciens,* which is cultured together with the callus. If the gene is successfully incorporated into the genome, there is a possibility of a transgenic plant differentiating from the callus.

* **Production of secondary metabolites:**

Suspension cultures are used in the production of usefull secondary metabolites. These are alkaloids, colourings, flavours, fragrances, terpenes etc, for the pharmaceutical, cosmetic and food industry. Large volumes are cultured in bioreactors that contain the suspension cultures under specific conditions, to produce high contents of the products. Shikonin, vinblastin, berberine and rosemarinic acid are some of these secondary metabolites that are produced by suspension culture on an industrial scale.

Anther culture:

Anther culture is the sterile isolation of anthers and development of haploid or doubled haploid callus cultures from microspores, from which plantlets can be differentiated. The technique is used for the rapid production of homozygotes in plant breeding programmes. Conventional breding programmes require over 8 generations after hybridization to produce homozygous lines. Homozygous lines would be available directly from F1 plants by culturing their anthers.



Anther culture is also useful in mutation breeding progranmmes. Anther culture enables the expession of recessive mutants in the first generation after mutagen treatment.

Protoplast culture:

Protoplast culture is the sterile isolation of cell protoplasts, with the goal of genetically modifying the cell. The cell wall is dissolved with enzymes to release the protoplasts.

***Somatic hybridization:**

Protoplast fusion is resorted to in wide crosses when hybridization is difficult. Isolated protoplasts are fused under suitable conditions to form a somatic hybrid. This is useful in introducing desirable genes such as disease resistance from wild species to crop plants.

***In producing transgenic plants**

DNA is directly injected to the protoplast by methods such as electroporation, micro-injection and microprojectile bombardment.

The protoplasts are next cultured in a suitable medium where they develop callus which can be induced to differentiate into plants incorporated with the foreign gene.

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 cultre 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. It is very essential to maintain cleanliness in all these rooms in order to reduce contamination of cultures by micro organisms.

EQUIPMENT IN A TISSUE CULTURE LABORATORY:-

Media preparation 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

4. Other organic substances:

   Amino acids, Vitamins, different nutrients of undefined composition such as coconut water, casein hydrolysate,Yeast extract etc.

5. Agar - It is added to modify the physical properties of the medium. It is an inert substance which gives a semi solid consistancy to the medium.

6. Activated charcoal - Adsorbs toxins produced by tissues

7. Acid/alkali - The pH of the medium is important for healthy growth. Generally a pH between 5.6 - 5.8 brings about optimum growth.

4. **Plant Growth Regulators:**

   Certain compounds occur naturally within plant tissue 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. There are incorporated in media to control growth as desired.

   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 growing tissues where there is cell division. Growth and differentiation in tissue cultures are regulated by the interaction and balance between growth regulators produced by the cultured cells and those incooporated in the medium.

The basic mechanisms in regulation in cells are 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 . Therefore the choice of a compound to be incorporated in the medium and its concentration 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 from the mother plant.
* 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.

eg. of Synthetic auxins,

* 2,4-dichlorophenozyacetic acid (2,4-D)
* alpha naphthaleneacetic acid (NAA)
* Indole-3- butyric acid (IBA)

Effects of auxins in tissue culture:

* Often brings about callus induction.
* Root induction
* Growth and elongation of meristem and shoot tips
* Somatic embryogenisis

Cytokinins:

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

eg. of Synthetic cytokinins

* Kinetin
* BAP (6-Benzylaminopurine)

Effects of cytokinins in tissue culture:

* Stimulation of cell division
* Axillary shoot proliferation
* Adventitous shoot formation
* Inhibition of root formation.

Composition of MS Medium (Murashige and Skoog 1962)

Compound	$mM\ l^{-1}$	$mg\ l^{-1}$
Macro nutrients		
$NH_4\ NO_3$	20.6	1650
KNO_3	18.8	1900
$CaCl_2.2H_2O$	3.0	440
$MgSO_4.7H_2O$	1.5	370
$KH_2\ PO_4$	1.2	170
Micro nutrients	$\mu M\ l^{-1}$	
$MnSO_4.\ 4H_2O$	100	22.3
$ZnSO_4.7H_2O$	30	8.6
H_3BO_3	100	6.2
KI	5	0.83
$Na_2\ MoO_4.2H_2O$	1.0	0.25
$CuSO_4.\ 5H_2O$	0.1	0.025
$CoCL_2.6H_2O$	0.1	0.025
Na_2EDTA	100	37.3
$FeSO_4.\ 7H_2O$	100	27.3
Organic compounds		
Thiamine. HCL	0.3	0.1
Nicotinic Acid	4.0	0.5
Pyridoxine	2.4	0.5
Glycine	26.6	2.0
Sucrose	876 mM	30,000.00