PLANT TISSUE CULTURE: AN OVERVIEW

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STRUCTURE

1.0. OBJECTIVES

After studying this unit you will be able to

- ✓ Discuss the importance of Tissue culture
- ✓ Define vocabulary related to plant tissue culture
- ✓ Describe the Totipotency capability of Plant Cells
- ✓ Describe the four steps of plant tissue culture
- ✓ Explain the benefits of plant tissue culture in agriculture

1.1. INTRODUCTION

In our previous chapters you have learnt about the different macro propagation methods of plant. Do you know plant can also be regenerated by using small pieces of plant tissue? The procedure of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid, under aseptic conditions is called Plant Tissue Culture.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones. The principal advantages of using tissue culture techniques is the rapid multiplication of plants which do not give seeds or have recalcitrant seeds, preservation of threatened and rare species of plants, production of plants with changed genotype (haploids, hybrids *etc.*), production of uniform clones from highly heterogeneous plants, production of disease free plant materials etc. There are many types of plant tissue culture techniques such as seed culture, embryo culture, shoot meristem culture, ovary or ovule culture, protoplast culture, suspension culture etc.

At present times numerous works devoted to plant tissue cultures proved how important this part of the plant science is. Follow the links to read more about this technique

https://www.frontiersin.org/articles/10.3389/fpls.2019.00536/full,

https://link.springer.com/article/10.1007/s11240-019-01724-1

1.2. CALLUS TISSUE AND ORGANOGENESIS

Callus (pl. calli) on a wounded plant parts or on a culture medium is made of an amorphous aggregate of loose parenchyma cells which proliferate from the mother cells. Callus is either homogenous parenchymatous mass or treachery elements or sieve elements or submerized cells or secretory cells or the trichomes. Callus contains no organized meristems. Callus is somewhat an abnormal tissue which has the potentiality to produce normal roots and embryoids and in turn it develops into plantlets.

Organogenesis is the development of adventitious organs or primordial (embryoid) from undifferentiated cell mass (callus) in tissue culture. It is controlled mostly by a balance between cytokinin and auxin (plytohormone). A relatively high ratio of auxin : cytokinin induces root formation in callus tissues whereas, a low ratio induces shoot formation.

When plants are multiplied vegetatively as distinguished from those grown from seeds whether by tissue culture or by cuttings, all the offspring from a single plant can be classified as a **clone**. This means that the genetic make-up of each offspring is identical to that of all the other offspring and to that of the single parent. On the other hand, plants propagated by seed, resulting from sexual reproduction, are not clones because each seed (and the resultant plant) has a unique genetic make-up-a mixture from two parents, different from either parent and different from one seed to another. The term **cloning**, with respect to tissue culture, refers to the process of propagating in culture large numbers of selected plants with the same genotype (the same genes or hereditary factors) as their respective parent plant.

Check Your Progress 1

i. In plant tissue culture, what is the term ORGANOGENESIS means?

a) Formation of callus culture
b) Formation of root and shoot from callus culture.
c) Genesis of organ
d) None of the above

ii. What is callus?

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1.3. WHY PLANTS REGENERATE?

Meristematic cells are located at the tips of stems and roots, in leaf axils, in stems as cambium, on leaf margins, and in callus tissue. Under the influence of genetic make-up, location, light, temperature, nutrients, hormones, and probably many other factors, meristematic cells differentiate into leaves, stems, roots and other organs and tissues in an organized fashion. Meristematic tissue is the basis of plant growth and development.

Parenchyma cells, the most common type of plant cell, are thin-walled cells that have the capacity to regenerate and differentiate, to initiate the growth of new and varied tissues or organs for specialized functions.

1.4. PROCESS INVOLVED IN PLANT TISSUE CULTURE TECHNIQUE

Plant tissue culture technique has developed around the concept that a plant cell is totipotent that it has the capacity and ability to develop into whole organism. There are some common stages present in the different plant tissue culture methods as follows:

1.4.1. Preparation of nutrient medium

A semi-solid medium is prepared in double distilled water containing macro elements, micro elements, amino acids, vitamins, iron source, carbon source like sucrose and phytohormones.

1.4.2. Establishment of aseptic culture

The starting material for the process is normally an actively growing shoot tip of auxiliary or terminal bud or shoot tip of a plant.

1.4.3. Inoculation

Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium.

1.4.4. Development of plant in growth room

After the inoculation of the plant tissue, the bottles are sealed and transferred into growth room to trigger developmental process under diffused light (fluorescent light of 10002000 lux) at 25 ± 2 C° and 50 to 60% relative humidity.

1.4.5. Hardening of micro plants

Due to very high humidity inside the culture vessel and artificial conditions of development, the plantlets re tender and are therefore are not ready for coping up with the field conditions.

1.4.6. Transfer to the Green house

Due to very high environmental fluctuations in the field, young developed plants can transfer to the green house for increasing its survival in the field. Green house have the less environment fluctuations as comparable to the field.

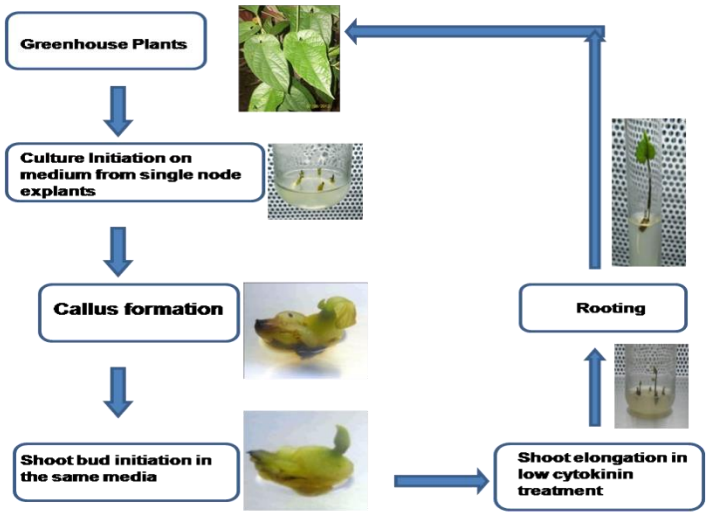

Figure : Schematic diagram of Plant Tissue Culture method

Check Your Progress 2

i. Which tissue is responsible for growth and development of plant?

a) Vascular tissue
b) Meristematic tissue
c) Sclerenchyma
d) Secretory tissue

ii. High cytokinin and low auxin induces _____

a) Shoot
b) Root
c) Germination
d) Organ

iii. What is totipotency?

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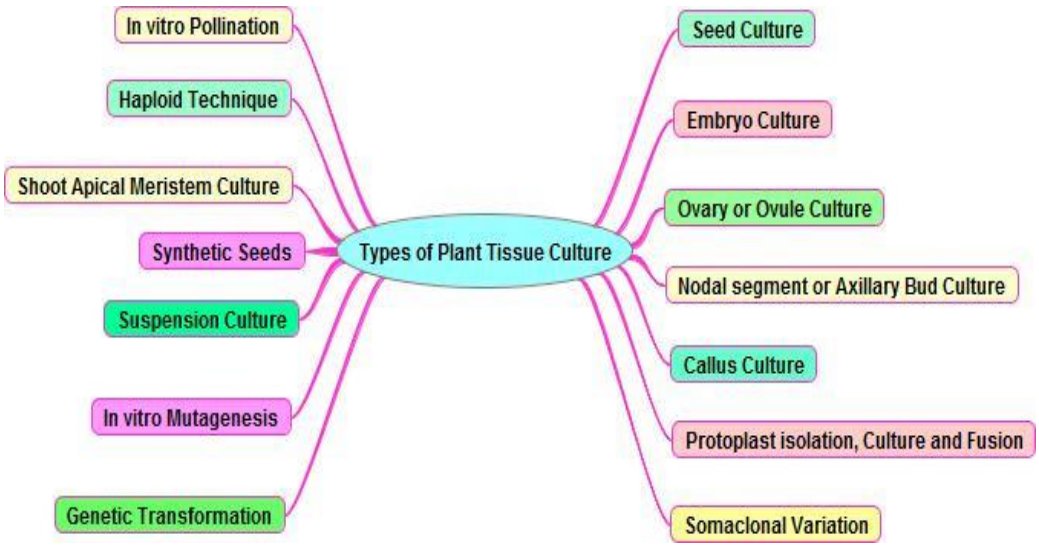
1.5. TYPES OF PLANT TISSUE CULTURE TECHNIQUES



1.5.1. Seed Culture

It is performed by surface sterilization and in vitro culture for increasing efficiency of germination of seeds which that are difficult to germinate in vivo.

1.5.2. Embryo Culture

Embryo culture is the sterile isolation and growth of an immature or mature embryo in vitro, with the goal of obtaining a viable plant. In plant breeding embryo culture have been valuable tools, especially for the transfer of biotic and abiotic resistance genes from wild relatives into crop plants.

1.5.3. In vitro pollination

When pollen is applied to stigma of ovaries cultured in vitro or directly onto ovules cultured with or without placental tissue, it is called in vitro pollination. Ovaries are collected from emasculated flowers usually 1-2 days after anthesis and cultured intact or with the ovarian, wall removed to expose the placenta. Alternatively, the entire placenta or pieces of placenta bearing ovules may be cultured.

1.5.4. Haploid Technique

Plant tissue culture have extended the range of crop species from which haploid plants have been produced as well as the efficiency resulting in large-scale haploid plant production by anther and microspore culture techniques. Specialized plant tissue culture methods have enabled the production of completely homozygous breeding lines from gametic cells in a shortened time frame compared to conventional plant breeding.

1.5.5. Ovary or ovule culture

In flowering plants, the ovule is typically located inside the ovary of the gynoecium which produces the ovules. Ovule consists of three parts: the integument(s) forming its outer layer(s), the nucellus (or megasporangium), and the megasporederived female gametophyte (or megagametophyte) in its center.

1.5.6. Shoot apical meristems culture

This is a method of asexual propagation used to produce clones of a particular plant in large quantities. The shoot apex explant measures between 100 to 500μm and includes the apical meristem with 1 to 3 leaf primordia.

1.5.7. Nodal segment or axillary bud culture

This consists of a piece of stem with axillary bud culture with or without a portion of shoot. When only the axillary bud is taken, it is designated as “axillary bud” culture. These techniques are mostly applied for mass propagation.

1.5.8. Synthetic seeds

Synthetic seeds (somatic embryo as substitutes for true seeds) can be produced either as coated or non-coated, desiccated somatic embryos or as embryos encapsulated in hydrated gel (usually calcium alginate). Successful utilization of synthetic seeds as propagules of choice requires an efficient

and reproducible production system and a high percentage of post-planting conversion into vigorous plants. Artificial coats and gel capsules containing nutrients, pesticides and beneficial organisms have long been thought as substitutes for seed coat and endosperm.

1.5.9. Callus cultures

Any explant i.e. any plant parts can be cultured to initiate callus. A callus is a mass of unorganized cells, which in many cases, upon transfer to suitable medium, is capable of giving rise to shoot buds and somatic embryos, which then form complete plants. In some instances it is necessary to go through a callus phase prior to regeneration via somatic embryogenesis or organogenesis.

1.5.10. Suspension Culture

Tissues and cells cultured in a liquid medium produce a suspension of single cells and cells clumps of few to many cells: these are called suspension cultures. Suspension cultures grow much faster than callus cultures need to be sub-cultured about every week, allow a more accurate determination of the nutritional requirements of cells and are amenable to scaling up for a large scale production of cells and even somatic embryos (SEs). The suspension cultures are broadly grouped as batch cultures, continuous cultures and immobilized cell cultures.

1.5.11. Protoplast isolation, culture and fusion

A protoplast is a cell that had its cell wall completely or partially removed using either mechanical or enzymatic means. Cell walls are made of a variety of polysaccharides. Protoplasts can be made by degrading cell walls with a mixture

of the appropriate polysaccharide-degrading enzymes.

1.5.12. In vitro mutagenesis

One of the applications of tissue culture systems is their exploitation for the induction and isolation of mutant cells, which can then be regenerated as mutant plants. While a number of mutations have been recognized in plant cells in vitro.

1.5.13. Somaclonal Variations

In plant breeding tissue culture in conventional micro propagation has resulted to a large extent in clonal fidelity, it has become increasingly clear

that under the appropriate culture conditions, a great deal of genetic variability can be recovered in regenerated plants. In early report, most of the variations were attributed to the readily detected chromosome instability of cultured plant cells. Reorganization of this spontaneous variation inherent in long- term culture led to the use of cell culture for mutagenesis and selection of genetic variants and for direct recovery of novel genotypes from cell cultures via somaclonal variation.

1.5.14. Genetic transformation

The ability to move DNA into an organism and thereby alter its genotype or genetic makeup is central to both basic and applied molecular biology. Genes derived from unrelated species and even other kingdoms, such as bacteria, fungi, plants, animals, that would otherwise be inaccessible to an organism, can be combined in the lab using genetic transformation techniques.

Check Your Progress 3

i. To obtain haploid plant, we culture
   a) Entire anther
   b) Nucleus
   c) Embryo
   d) Apical bud

ii. What is suspension culture?

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iii. Write the difference between callus culture and suspension culture

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1.6. IMPORTANCE OF TISSUE CULTURE TECHNIQUE

i. Germination of seeds which that are difficult to germinate.

ii. Make wide crosses with a greater number of related species of wild plants and have access to a much wider range of genes that can be used for genetic improvement of crop plants.
iii. Shortening of breeding cycle culturing immature embryos especially in marker assisted selection (MAS).
iv. Overcoming seed dormancy, self-sterility of seeds and embryo abortion due to incompatibility barriers.
v. Embryo rescue in distant (inter-specific or intergeneric) hybridization where endosperm development is poor.
vi. Production of homozygous diploid lines through chromosome doubling thus is reducing the time required to produce inbred lines.
vii. Developing "Double Haploid (DH)" mapping populations for QTL (Quantitative Trait Loci) analysis.
viii. Unfertilized ovary and ovule culture may lead to production of haploid plants
ix. Overcoming abortion of embryos of wide hybrids at very early stages of development due to incompatibility barriers. Mass in vitro propagation for plantation, virus free plant and desirable genotypes.
x. Facilitation of germplasm exchange between locations (production of clean material) and Cryopreservation (cold storage) or in vitro conservation of germplasm.
xi. One of the major pathway of regeneration and production of artificial seeds.
xii. For generation of useful somaclonal variants (genetic or epigenetic), production of metabolites and in vitro selection.

1.7. SUMMARY

In this unit you have learnt that

- Plant tissue culture refers to a method in which fragments of a tissue are introduced into a new, artificial environment, where they continue to function or grow.

- Plant tissue culture works on the basis of totipotency. It is the ability of a plant cell to form the complete plant through dedifferentiation and redifferentiation.

- Plant tissue culture involves different steps like establishment of aseptic culture, inoculation, development of plantlets, hardening and transfer of plantlets to green house.

- The rapid production of high quality, disease free and uniform planting stock is only possible through plant tissue culture.

- Plant tissue culture may be used for cloning purposes, genetic modification of a given plant or simply to accelerate or increase yield of the plant of interest.

1.8. TERMINAL QUESTIONS

i. What is plant tissue culture?

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ii. Briefly describe the hardening process of plant tissue culture.

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iii. List some importance of plant tissue culture

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1.9. ANSWERS

Check Your Progress 1

i. b

ii. Callus is an amorphous aggregate of loose parenchyma cells which proliferate from the mother cells.

Check Your Progress 2

i. b

ii. a

iii. **Totipotency** is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of totipotent cells.

Check Your Progress 3

i. a

ii. Refer Sec. 1.5.10

iii. Refer Sec. 1.5.9. and 1.5.10

Terminal Questions

i. The procedure of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid, under aseptic conditions is called Plant Tissue Culture.

ii. Refer Sec. 1.4.5 and 1.4.6.

iii. Refer Sec. 1.6

1.10. SUGGESTED READINGS

Gupta, P, K. (2008). *Element of Biotechnology*. Rastogi Publications. Meerut (U.P).

Kumar, U. (2007). *Methods in Plant Tissue Culture*. Agrobios (India) Publication. Jodhpur

Smith, R. H. (2006). *Plant Tissue Culture: Techniques and Experiments*. Elsevier.

1.11. REFERENCES

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Mohit and Sirohi, S. P. S. (2018). *Plant tissue culture techniques in crop improvement.* Internation Journal of Current Research. 10 (10). 74067-74070.