Topic: General Account of Tissue Culture
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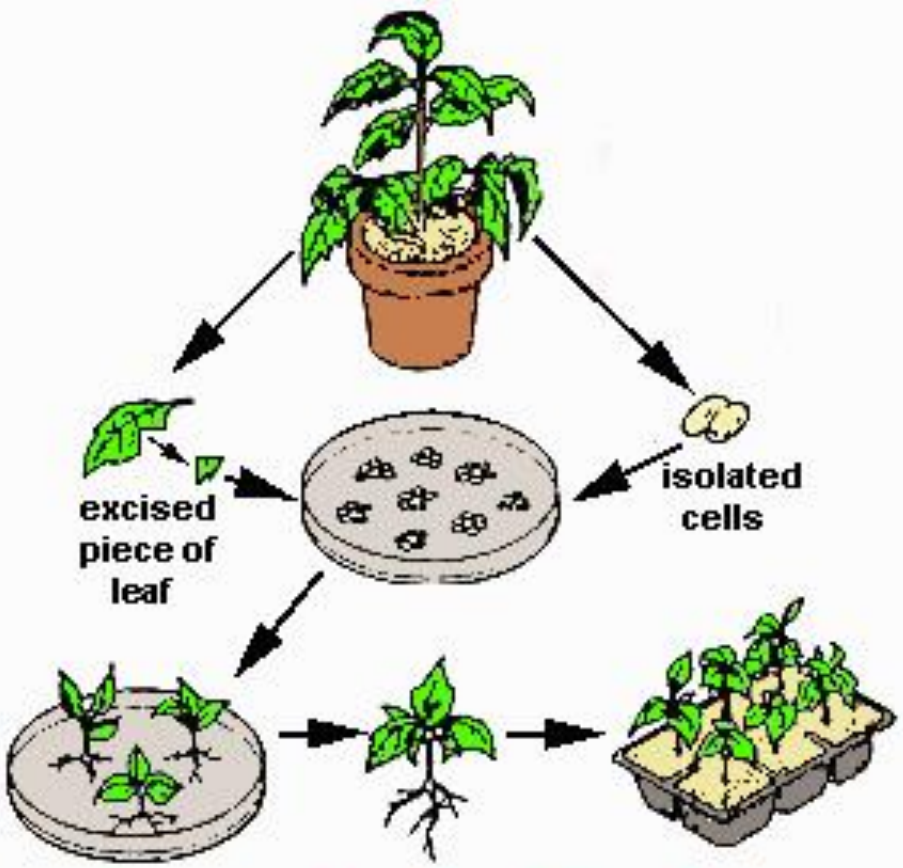
The science of plant tissue culture takes its roots from the discovery of cell followed by propounding of cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic structural unit of all living organisms. They visualized that cell is capable of autonomy and therefore it should be possible for each cell if given an environment to regenerate into whole plant.

Based on this premise, in 1902, a German physiologist, Gottlieb Haberlandt for the first time attempted to culture isolated single palisade cells from leaves in knop's salt solution enriched with sucrose. The cells remained alive for up to one month, increased in size, accumulated starch but failed to divide. Though he was unsuccessful but laid down the foundation of tissue culture technology for which he is regarded as the father of plant tissue culture.

In India, the work on tissue culture was initiated during 1950s at University of Delhi. This initiation is credited to P. Maheshwari who was working there in the Department of Botany. Discovery of haploid production was a land-mark in the development of in-vitro culturing of plants.



Overview of the Tissue Culture Process

Plant tissue culture has a great significance in plant biotechnology specially in the crop improvement programmes. The term tissue culture may be defined as the process of in-vitro culture of explants (pieces of living differentiated tissues) in nutrient medium under aseptic conditions. However, in general, the tissue culture includes the term tissue culture as well as cell culture, organ culture and suspension culture also.

Plant tissue culture is fundamental to most aspects of biotechnology of plants. It is evident now that plant biotechnology is one of the most beneficial of all the sciences. The products of plant biotechnology are being transferred rapidly from laboratories to the fields.

Also, the plant tissue culture has become of great interest to the molecular biologists, plant breeders and even to the industrialists, as it helps in improving the plants of economic importance. In addition to all this, the tissue culture contributes

immensely for understanding the patterns and responsible factors of growth, metabolism, morphogenesis and differentiation of plants.

Basic Requirements and Techniques of Plant Tissue Culture:

The main requirements of plant tissue culture are:

- Laboratory organization
- Culture Media
- Aseptic Conditions

The most popular and advantageous methods in plant tissue culture are discussed below:

1. Cell Culture:

Cell culture is actually, the process of producing clones of a single cell. The clones of cell are the cells which have been derived from the single cell through mitosis and are identical to each other as well as to parental cell. First attempts for cell culture were made by Haberlandt in 1902. However, he failed to culture single cell but his attempts stimulated other workers to achieve success in this direction.

The method of cell culture is meritorious over other methods of culturing because it serves as the best way to analyse and understand the cell metabolism and effects of different chemical substances on the cellular responses. Single cell culturing is of immense help in crop improvement programmes through the extension of genetic engineering techniques in higher plants.

The method of cell culture is done by following three main steps:

(a) Isolation of single cell from the intact plant by using some enzymatic or mechanical methods.

(b) In-vitro culturing of the single cell utilizing micro chamber technique, or micro drop method or Bergmann cell plating technique.

(c) Testing of cell viability done with the phase contrast microscopy or certain special dyes.

It is important to note here that the cell cultures require a suitably enriched nutrient medium and it should be done in dark because light may deteriorate the cell culture.

Large scale culturing of plant cells under in-vitro conditions provides a suitable method for production of large varieties of commercially important phytochemicals.

2. Suspension Culture:

A culture which consists of cells or cell aggregates initiated by placing callus tissues in an agitated liquid medium is called as a suspension culture. The continuous agitation of the liquid medium during a suspension culture is done by using a suitable device called as shaker, most common being the platform/orbital shaker.

Agitation with shaker is important because it breaks the cell aggregates into single cell or smaller groups of cells and it helps in maintaining the uniform distribution of single cell and groups of cells in the liquid medium.

A good suspension is the one which has high proportion of single cells than the groups of cells. Changes in the nutritional composition of medium may also serve as a useful technique for breakage of larger cell clumps.

The general technique of suspension culture involves basically two types of cultures: batch culture and continuous cultures.

A batch culture is a suspension culture in which cells grow in a finite volume of the culture medium and as a result, medium gradually depletes. On the other hand, a continuous suspension culture is the one which is continuously supplied with nutrients by the inflow of fresh medium but the culture volume is normally constant.

3. Root Culture:

Pioneering attempts for root culture were made by Robbins and Kotte during 1920s. Later on, many workers tried for achieving successful root cultures. In 1934, it was White who successfully cultured the continuously growing tomato root tips.

Subsequently, root culturing of a number of plant species of angiosperms as well as gymnosperms has been done successfully. Root cultures are usually not helpful for giving rise to complete plants but they have importance's of their own. They provide beneficial information regarding the nutritional needs, physiological activities, nodulations,

infections by different pathogenic bacteria or other microbes, etc.

4. Shoot Culture:

Shoot cultures have great applicability in the fields of horticulture, agriculture and forestry. The practical application of this method was proposed by Morel and Martin (1952) after they successfully recovered the complete Dahalia plant from shoot-tips cultures.

Later on, Morel realized that the technique of shoot culturing can prove to be a potent method for rapid propagation of plants (i.e. Micro propagation). In this technique, the shoot apical meristem is cultured on a suitable nutrient medium. This is also referred to as Meristem Culture

The apical meristem of a shoot is the portion which is lying beyond the youngest leaf primordium. Meristem tip culture is also beneficial for recovery of pathogen-free specially virus-free plants through the tissue culture techniques. Various stages in this culture process are the initiation of culture, shoot multiplication, rooting of shoots and finally

the transfer of plantlets to the pots or fields.

5. Protoplast Culture:

A protoplast is described as a plasma membrane bound vesicle which consists of a naked cell formed as a result of removal of cell wall. The cell wall can be removed by mechanical or enzymatic methods. In-vitro culturing of protoplasts has immense applications in the field of plant biotechnology.

It not only serves for genetic manipulations in plants but also for biochemical and metabolic studies in plants. For protoplast culture, firstly the protoplasts are isolated from the plants utilizing some chemical or enzymatic procedure.

At present, there are available a number of enzymes which have enabled the isolation of protoplasts from almost every plant tissue. After isolation of protoplasts, they are purified and then tested for their viability. Finally the purified viable protoplasts are cultured in-vitro using suitable nutrient medium which is usually either a liquid medium or a semisolid agar medium.

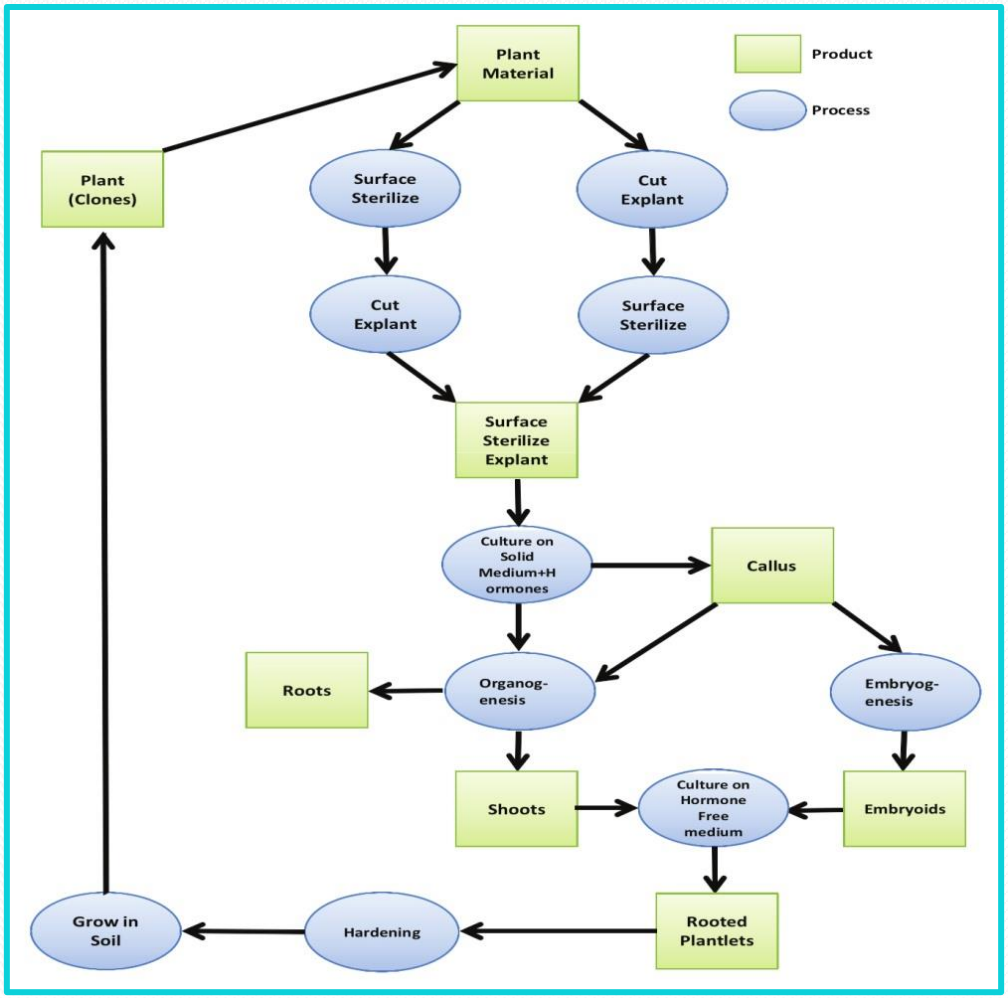


Fig. Schematic representation of production of hybrid plant via protoplast fusion

6. Haploid Production:

Haploid plants are those which contain half the number of chromosomes (denoted by n). Haploids can be exploited for benefits in the studies related to experimental embryogenesis, cytogenetics and plant breeding. Haploids have great significance in field of plant breeding and genetics. They are most useful as the source of homozygous lines.

In addition, the in-vitro production of haploids also aids for induction of genetic variabilities, disease resistance, salt tolerance, insect resistance, etc. Presently, attention is being focused on improving the frequencies of haploid production in their advantageous utilization for economic plant improvement.

There are two approaches for in-vitro haploid production are-

(a) Androgenesis:

The technique of production of haploids through anther or microspore culture is termed as androgenesis. It is a method par excellence for the large scale production of haploids through tissue culture.

Androgenesis technique for haploid production is based on the in-vitro culture of male gametophyte i.e., microspore of a plant resulting into the production of complete plant from it. It is achieved either by another culture or by microspore (pollen) culture.

(b) Gynogenesis:

It is an alternative source of in-vitro haploid production. It refers to the production of haploid plant from ovary culture or ovule culture. The method of gynogenesis for haploid production has been successful, so far, in a very few plants only, hence it is not a very popular method for in-vitro production of haploids. Thus, androgenesis is preferred over gynogenesis.

7. Embryo Culture:

The technique of embryo culture involves the isolation and growth of an embryo under in-vitro conditions to obtain a complete viable plant. First success for embryo culture was made by Hannig in 1904 when he isolated and cultured embryos of two crucifers namely Cochleria and Raphanus. Embryo culture is used widely in the fields of

agriculture, horticulture and forestry for production of hybrid plants.

This technique allows the detailed study about the nutritional requirements of embryos during different developmental stages. Also, it helps for identifying the regeneration potential of embryos. Embryo culture is advantageous for in-vitro micro propagation of plants, overcoming seed dormancy and for production of beneficial haploid plants.

8. Endosperm Culture (Triploid Production):

Endosperm tissue is triploid therefore the plantlets originating by the culture of endosperm are also triploid.

In majority of flowering plant families (exceptions being Orchidaceae, Podostemaceae, Trapaceae which lack endosperm) the endosperm tissues are present. Endosperm is formed after the double fertilization of one male nucleus with two polar nuclei. Immature endosperm has more potential of growth in culture especially among the cereals.

Endosperm culture has provided a novel strategy for plant breeding and horticulture

for the production of triploid plantlets. It is an easy method for production of a large number of triploids in one step.

Moreover, it is much more convenient that the conventional techniques like chromosome doubling by crossing tetraploids with diploids for triploid induction. Full triploid plants of endosperm origin have been produced in a number of plant species like *Populus, Oryza sativa, Emblica officinalis, Pyrus malus, Prunus*, etc.

The triploid plants are usually seedless therefore this technique is most beneficial for increasing the commercial value of fruits like apple, mango, grapes, watermelon, etc. In addition to all the above described applications, endosperm culture is helpful for studying biosynthesis and metabolism of certain natural products also.

Applications of Plant Tissue Culture:

- Germplasm conservation mainly in the form of cryopreservation of somatic embryos or shoot apices, etc.
- Large scale production of useful compounds and secondary metabolites by using

genetically engineered plant tissue cultures.

- Technique of micro propagation for enhancing the rate of multiplication of economically important plants.
- Eradication of systemic diseases in plants and raising disease free plants.
- Soma-clonal variations are useful sources of introduction of valuable genetic variations in plants.
- Helps plants in imparting resistance to antibiotics, drought, salinity, diseases, etc.
- Somatic hybrids and cybrids overcome species barriers and sexual incompatibility and produce hybrid plants with desired combination of traits.
- Embryo culture helps in overcoming seed sterility and dormancy.
- Haploid production in culture helps to solve various problems of genetic studies and thus aids the plant breeders for producing new varieties.
- Production of synthetic seeds via somatic embryo differentiation for commercially important plants helps to achieve increased agricultural production.

- Large scale production of biomass energy.
- Plant tissue culture aids in producing the genetically transformed plants.
- Early flowering can be induced by in-vitro culturing of plants so as to attain commercial benefits.
- Triploids as well as polyploid plants can also be produced by tissue culture techniques for uses in plant breeding, horticulture and forestry.
- Seedless fruits and vegetables can be produced by following the endosperm culture method which add to their commercial values.
- Increased Nitrogen fixation ability can be achieved through association of tissue culture techniques with genetic engineering.
- Callus cultures are useful in plant pathology as they act as an effective tool in the study of mechanism of disease resistance and susceptibility.
- Different tissue culture techniques help us to study various biosynthetic processes, physiological changes and cytogenetic changes.