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Plant Tissue Culture Technique and Potential Application

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ARTICLE ID: 64

Introduction

Plant tissue culture is a set of techniques used to preserve or expand plant cells, tissues or organs under sterile conditions in a nutrient culture medium of defined composition.Plant tissue culture depends on the ability of plant tissue, if provided with a growth medium and suitable environment, to give rise to a whole new plant. This capacity of plant cells or tissues is called 'totipotency.'

Requirements for plant tissue culture:

1. A suitable explant
2. A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid
3. Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can overrun a culture
4. Growth regulators in plants, both auxins andcytokinins

Stages of Plant tissue culture:

1. Zero stage: Selectionof healthy parent plants known as explants
2. First stage: Establishment of explants
3. Second stage: Shooting of explants
4. Third stage: Rooting of plants
5. Fourth stage: Hardening and acclimatization

Generalized procedure of plant tissue culture

1. **Selection of explants**:

The tissue obtained from a plant to be cultured is called an explant.In many species explants of various organs vary in their rates of growth and regeneration. The choice of

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explant material also determines if the plantlets developed via tissue culture are haploid or diploid.

2. **Establishment of explants**:

Before establishment explants are surface sterilized. Therefore, surface sterilization is essentially the method of keeping explants free of contaminants before cultures are esta blished. It is essential that the explants be free of any contaminants including endophytic, prior to tissue culture without losing their biological activity. Surface sterilization with 70% ethanol for 1 min followed by 1% sodium hypochlorite (NaOCI) (+ 2-3 drops of Tween 20) for 15 min proved most effective for maximum survival percentage in leaf explants whilst 70% ethanol washing for 2 min, followed by 0.1% mercuric chloride (HgCl2) for 5 min proved to be more effective for maximum survival percentage in nodal explants. After surface sterilization, explants plants are cultured on MS (Murashige and Skoog) basal media for establishment. And then, cultures are incubated in culture room under optimal conditions of light, temperature, humidity, and photoperiod etc.After few days, when proliferation appears, plants are subjected to multiplication media provided with various combinations of growth regulators.

3. **Multiplication**:

The goal of the shoot multiplication phase is to increase the number of shoots produce d by the initial explant. The number of shoots produced in culture increases significantly by subculturing these new shoots on to a new medium. In order to establish a new plantlet, shoots multiplied by culture must be rooted in Stage III.

4. **Rooting**:

Microcuttings are induced to form roots at the rooting stage-usually through the use of auxin.

5. **Hardening and Acclimatization**:

Finally, once the roots have been well formed on microcutting, the plantlets must be acclimatized to a normal growing climate in stage IV.This involves progressively moving to open-air conditions where the humidity is lowered and the light levels increased.

Milestones in Plant Tissue Culture

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- **Haberlandt (1902):** Haberlandtcultured plant cells in an artificial condition called *in vitro* (inside glass) in culture medium (Knop's salt solution) consisting of glucose and peptone and developed callus (unorganized mass of cells) and proposed the concept of 'totipotency',which

  involves the *in vitro* production of the whole plant from isolated cells or tissue.
- **P.R.White (1934):** P.R. White used Knop's solution along with three vitamins like pyridoxine, thiamine and nicotinic acid and developed root cultures.
- **F.C. Steward (1948):** F.C. Stewardused coconut water in plant tissue culture and obtained cell proliferation from carrot explants.
- **Morel and Martin (1952, 1955):** Morel and Martindeveloped virus-free Dahlia and potato plants using shoot meristem culture.
- **Murashige and Skoog (1962):** The tissue culture medium formulated by Murashige and Skoog is a pioneer of plant tissue culture and is the most widely used medium for all forms of work of tissue culture.
- **Kanta et al. (1962):**Theydeveloped test-tube fertilization in flowering plants.
- **Yamada et al. (1963):** They developed calli and free cells in the tissue culture of *Tradescantia reflexa*.
- **Guha and Maheshwari (1964):**Theydeveloped *in vitro* production of haploid embryos from anthers of *Datura innoxia*.
- **Vasil and Hildbrandt (1965):** They achieved differentiation of tobacco plants from single, isolated cells in micro propagation.
- **Takebe et al. (1971):**regenerated tobacco plants from isolated mesophyll protoplasts.
- **Carlson**and co-workers obtained protoplast fusion between Nicotiana glauca and Nicotiana longsdorffii and developed first interspecific somatic hybrid in 1971.
- **Melchers and co-workers in 1978:** developed intergenic hybrid between potato and tomato called pomato.
- **Chilton (1983):**produced transformed tobacco plants from single cell transformation and gene insertion.
- **Horsh et al. (1984):**developed transgenic tobacco by Agrobacterium mediated gene transfer.

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Potential Applications of plant tissue culture

1. **Tissue culture in agriculture**

   As an evolving technology, the plant tissue culture has a great influence on both agriculture and industry, by supplying plants needed to satisfy the ever-global demand. It has made important contributions to the development of agricultural sciences in recent times and today they constitute an essential method in modern agriculture.

   a. Production of improved crop varieties
   b. Production of disease-free plants (virus)
   c. Genetic transformation
   d. Production of secondary metabolites
   e. Production of varieties tolerant to salinity, drought and heat stresses

2. **Germplasm conservation**

In vitro cell and organ culture provides an alternate source for the conservation of endangered genotypes. Owing to the high rate of extinction of plant species and the enhanced need to conserve the floristic heritage of nations, germplasm preservation is rapidly becoming an important task worldwide. When the aims for conservation are clones instead of plants, tissue culture protocols may be used to protect vegetative tissues, to retain the genetic history of a crop and to prevent the depletion of preserved heritage due to natural disasters, whether biotic or abiotic stress. Cryopreservation plays a vital role in the long-term *in vitro* conservation of essential biological material and genetic resources. It requires the preservation of *in vitro* cells or tissues in liquid nitrogen that leads to cryo-injury on the exposure of tissues to physical and chemical stresses.Cryobionomics is a modern method to study genetic stability in the cryopreserved plant materials. The embryonic tissues may be cryopreserved for potential use or for

3. **Embryo culture**

   Embryo culture is a form of plant tissue culture that is used to develop embryos from seeds and ovules in a nutrient medium. In embryo culture, the plant develops directly from the embryo or indirectly via callus formation and then subsequent development of shoots and roots. The technique has been developed to break seed dormancy,test the viability of seeds, development of rare species and haploid plants. It is an effective

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strategy that is employed to shorten the breeding cycle of plants by developing excised embryos and results in the reduction of long dormancy period of seeds. With the basic objective of mass multiplication, intra-varietal hybrids of the economically essential energy plant 'Jatropha' were successfully developed. An effective protocol has been developed for the *in vitro* propagation of Khayagrandifoliola by excising embryos from mature seeds. The plant has a high commercial importance for timber wood and medicinal purposes.

4. **Genetic transformation:**

The most recent feature of plant cell and tissue culture is genetic transformation, which includes the means of transferring genes with a favourable characteristic into host plants and recovery of transgenic plants. The technique has a great potential of genetic enhancement of different crop plants by incorporating in plant biotechnology and breeding programmes. It has a promising role for the introduction of agronomically important traits such as increased yield, improved quality and enhanced resistance to pests and diseases. Researchers succeeded in creating transgenic plants of potato resistant to potato virus Y (PVY) which is a big challenge to potato crop worldwide.

5. **Protoplast fusion:**

Somatic hybridization, through the development of interspecific and intergeneric hybrids, is an important plant breeding and crop improvement technique.The technique involves the fusion of protoplasts of two distinct genomes followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants. Protoplast fusion provides an effective means of gene transfer with desired trait from one plant to another and has a growing effect on crop improvement. Somatic hybrids were developed using electrofusion treatment for salt tolerance by fusion of rice and ditch reed protoplasts.

6. **Haploid production:**

The tissue culture techniques allow the development of homozygous plants through the protoplast, anther and microspore cultures in a relatively short period of time instead of traditional breeding methods. Haploids are sterile plants with a single set of chromosomes that, through random or mediated chromosome doubling, are transformed into homozygous diploids. Doubling of chromosomes enhances plant fertility, resulting in the development of double haploids with the ability to become pure breeding new

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cultivars. The term androgenesis refers to the development of haploid plants from young pollen cells without undergoing fertilization. Introduction of genes with desired trait at haploid state followed by chromosome doubling led to the development of double haploids inbred wheat and drought resistant plants were attained successfully.

Other applications of plant tissue culture

- Improved hybrids production through somatic hybridization.
- Somatic embryoids can be encapsulated into synthetic seeds (synseeds). These encapsulated seeds or synthetic seeds help in conservation of plant biodiversity.
- Production of disease resistant plants through meristem and shoot tip culture.Meristem tip culture, can be used to produce safe, virus-free plant material, such as sugar cane, potatoes and other soft fruits.
- Production of stress resistant plants like herbicide tolerant, heat tolerant plants.
- Micro propagation technique to obtain large numbers of plantlets of both crop and tree species useful in forestry within a short span of time and all through the year.
- Production of secondary metabolites from cell culture utilized in pharmaceutical, cosmetic and food industries.