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RESEARCH ARTICLE

PLANT TISSUE CULTURE TECHNIQUES IN CROP IMPROVEMENT

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ABSTRACT

Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile conditions. Many primary steps involved are the preparation of nutrient medium, the establishment of aseptic culture, inoculation, development of the plant in growth room, hardening of micro plants and transfer to the greenhouse. Plant tissue culture has a lot of applications in crop improvement like making successful distant crosses, shortening breeding cycles, somaclonal variants, germplasm exchange 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., Therefore in this article an attempt has been made to give a brief idea about the various plant tissue culture techniques and their applications.

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INTRODUCTION

Plant tissue culture is a technique of *in vitro* cultivation of plant cells and organs, which divide and regenerate into callus or particular plant organs. In 1902, a German physiologist, Gottlieb Haberlandt developed the concept of *in vitro* cell culture. He isolated single fully differentiated individual plant cells from different plant species like palisade cells from leaves of *Laminum purpureum*, glandular hair of *Pulmonaria* and pith cells from petioles of *Eicchornia crassiples* etc and was first to culture them in Knop's salt solution enriched with glucose. In his cultures, cells increased in size, accumulated starch but failed to divide. Therefore, Haberlandt's prediction failed that the cultured plant cells could grow, divide and develop into embryo and then to whole plant. This potential of a cell is known as totipotency, a term coined by Steward in 1968. Despite lack of success, Haberlandt made several predictions about the requirements in media in experimental conditions which could possibly induce cell division, proliferation and embryo induction. G. Haberlandt is thus regarded as father of tissue culture.

Physical Components of Tissue Culture Technology

The basic equipment in most tissue culture facilities includes the following:

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Autoclave: An autoclave is basically a large-sized but sophisticated pressure cooker, and is used for the sterilization of the medium, glassware and instruments. Autoclaves of different sizes are available commercially. High-pressure heat is needed to sterilize media, water, and glassware. Certain spores from fungi and bacteria are killed only at 121^0 C and 1.05kg/sq.cm (15 pounds per sq. inch) pressure.

Laminar airflow chamber: The laminar flow chambers provide clean filtered air that allows cultures to be handled under contamination-free environment. The laminar-flow cabinets are located in the culture transfer area.

Other equipment: The sterilization of instruments, such as the forceps, scalpel holders and blades is achieved with gas flamed burner. The medium preparation room usually has the following equipment. A refrigerator-freezer to store chemicals and stock solutions, weighing scales for large amounts of over 10 g, and analytical balance with1 mg accuracy, a magnetic stirrer for the agitation, and a pH meter. An aspirator can be attached to a water tap for filter sterilization of chemicals and for surface sterilization of the plant material. A drying oven is required to keep glassware such as beakers, flasks and cylinders, and is also useful for dry sterilization of scalpels and glassware, such as Petri dishes, pipettes and others.

Non-essential equipment: Microwave ovens are convenient for defrosting stock solutions and pre-heating agar media. Laboratory glassware washers or regular dishwashers can be used for replacing manual work. Automatic media dispensers

are helpful to pipette pre-set volume of media. A gyratory shaker or a reciprocal shaker is necessary if micro-propagation is based on liquid media or suspension cultures. Computers, photocopiers and fax machines are helpful for easy data management and maintenance of records.

Activity specific requirements: Based on the different activities of a tissue culture, a facility can be divided into semi-clean, clean and ultra-clean areas. The semi-clean areas comprise of the washing room, office and staff restrooms, where there is no need for maintaining sterile conditions. The clean areas encompass the media preparation and sterilization rooms, which have to be sufficiently clean. High sterility has to be maintained in the culture transfer rooms and the growth rooms, which constitute the ultra-clean areas.

Glassware washing and storage area: The glassware washing area should be located near the sterilization and medium preparation rooms. Both hot and cold water should be available and the water still and de-ionization unit should be located nearby. Ovens or hot air-cabinets should be located close to the glassware washing and storage area. Dust-proof cabinets and storage containers should be installed to allow for easy access to glassware.

The Store: It is advisable to have a separate area for storage of chemicals, apparatus and equipment**.**

Media preparation and sterilization area: The media preparation room should have smooth walls and floors, which enable easy cleaning to maintain a high degree of cleanliness. The media preparation and sterilization can be carried out in the same area but preferably in different rooms, which need not be separated with doors. It is essential to have safety devices like fire extinguisher, fire blanket and a first aid kit in the media preparation room. A variety of glassware, plastic ware and stainless steel apparatus is required for measuring, mixing, and media storage.

Sterilization room: The sterilizing room should be in continuation with the media preparation room. The sterilization room must have walls and floors that can withstand moisture, heat and steam. For large volume media making, horizontal or vertical autoclaves should be installed.

Transfer room: The most important work area is the culture transfer room where the core activity takes place. The transfer area needs to be as clean as possible and be a separate room with minimal air disturbance. Fire extinguishers and first aid kits should be provided in the transfer room as a safety measure. The personnel should leave shoes outside the room. Special laboratory shoes and coats should be worn in this area.

Growth room: Growth room is an equally important area where plant cultures are maintained under controlled environmental conditions to achieve optimal growth. Lights directly fitted to the racks create uneven heat distribution. This leads to high humidity within the culture containers, which in turn can cause hyperhydricity. Sideways illumination is an alternative, which requires less number of lights, and provides more uniform lighting. Temperature in the growth room is usually controlled with air conditioners. Generally, temperatures are kept around 22^0C. Some plant cultures can be kept in complete darkness; however, most culture rooms need to be illuminated at 1 Klux [134.5 µmole/m2/s (microeinsteins per second per sq. centimeter or approximately 1076 foot candle) with some up to 5 to 10 Klux (672-1345 µmole/m2/s). The developmental stage of the plants also determines if wide spectrum or cool white-fluorescent lights are to be used. Shelves within the growth rooms vary depending upon the situation and the plants grown. Frames for the shelves can be made from 1.25cm (half-inch) thick angle iron.

Greenhouse facility: A critical stage in plant tissue culture is the interim phase between the laboratory and field conditions. *In vitro* derived plants need to be gradually hardened to field conditions. Plant hardening is usually carried out under greenhouse that ensures high survival of the tissue-cultured plants in the field. Appropriate light, shading and blackout systems can be achieved with supplementary lighting.

Process involved in Plant Tissue Culture Technique

There are some common stages present in the different plant tissue culture methods as follows:

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 phyto-hormones.
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.
3. *Inoculation:* Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium.
4. **Development of plant in growth room:** After the inoculation of the plant tissue, the bottles are sealed and transfer red into growth room to trigger developmental process under diffused light (fluorescent light of 1000-2000 lux) at 25 ± 2 o Cand 50 to 60% relative humidity.
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.
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.

Types of Plant Tissue Culture Techniques

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.
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.
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.

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.
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 megaspore-derived female gametophyte (or megagametophyte) in its center.
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.
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.
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.
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.
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.
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.
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.*
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.
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.

Applications of Plant Tissue Culture in Crop Improvement

- Germination of seeds which that are difficult to germinate.
- 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.
- Shortening of breeding cycle culturing immature embryos especially in marker assisted selection (MAS).
- Overcoming seed dormancy, self-sterility of seeds and embryo abortion due to incompatibility barriers.
- Embryo rescue in distant (inter-specific or inter-generic) hybridization where endosperm development is poor.
- Production of homozygous diploid lines through chromosome doubling, thus reducing the time required to produce inbred lines.
- Developing "Double Haploid (DH)" mapping populations for QTL analysis.
- Unfertilized ovary and ovule culture may lead to production of haploid plants
- 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.
- Facilitation of germplasm exchange between locations (production of clean material) and Cryopreservation (cold storage) or *in vitro* conservation of germplasm.
- One of the major pathway of regeneration and production of artificial seeds.
- For generation of useful somaclonal variants (genetic or epigenetic), production of metabolites and *in vitro* selection.

- Combining genomes to produce somatic hybrids, asymmetric hybrids or cybrids, plants that show physical or chemical incompatibility in normal sexual crosses, may be produced by the fusion of protoplasts obtained from two cultures of different species and cybridization especially for transfer of cytoplasmic male sterility.
- Widely used for DNA transformation (for making genetically modified organisms), since the cell wall would otherwise block the passage of DNA into the cell.
- Introduction of foreign DNA to generate novel (and typically desirable) genetic combinations and used to study the function of genes.

Conclusion

Tissue culture is important part of applied biotechnology. In the coming decades the world's population will increases more and accommodation space, agricultural lands will decrease significantly global climate change is also another consideration. Keeping these in mind we have to ensure a peaceful, healthy and hunger free greener world for our next generation. For doing this there is no alternate of plant tissue culture. Plant tissue culture is now a well established technology which has made significant contributions to the propagation and improvement of agricultural crops.

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