Introduction

What is Plant Tissue Culture?

Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (in vitro). It has applications in research and commerce. In commercial settings, tissue culture is primarily used for plant propagation and is often referred to as micropropagation. Micropropagation refers to the production of whole plants from cell cultures derived from explants (the initial piece of tissue put into culture); the explants usually consist of tissues that contain or develop into meristem cells.

What conditions do plant cells need to multiply in vitro?

Freedom from competition

Many early tissue culture experiments failed, at least in part, because they were not maintained in sterile conditions. Isolated fragments of a plant are extremely disadvantaged in comparison to pathogenic competitors that are complete and unhindered, in reality flourishing, in a culture environment. Bacteria, fungi, and other organisms which can be resisted to some degree by a whole plant can easily outcompete an isolated fragment of tissue from the plant in the relatively nutrient-rich environment of a culture flask. Therefore it is necessary to remove competitor organisms from the culture and isolate it in aseptic conditions. This is usually done by chemical surface sterilization of the explant with an agent such as bleach at a concentration and for a duration that will kill or remove pathogens without injuring the plant cells beyond recovery. The medium and culture flasks used must also be sterile.

Nutrients, proper hormones, and removal of waste products

When a small portion of a plant is isolated, it is no longer able to receive nutrients or hormones from the plant, and these must be provided to allow growth in vitro. The composition of the nutrient medium is for the most part similar, although the exact components and quantities will vary for different species and purpose of culture. Types and amounts of hormones vary greatly. In addition, the culture must be provided with the ability to excrete the waste products of cell metabolism. This is accomplished by culturing on or in a defined culture medium, which is periodically replenished.

A Controlled Environment

Tissue cultures, sustained by the nutritive medium and confined in a protective vessel, require a stable and suitable climate. Thus light and temperature must be more carefully regulated than would be the case for a whole plant.

Why is Tissue Culture Done?

Tissue culture offers numerous significant benefits over traditional propagation methods:

- Propagation can be much more rapid than by traditional means
- It may be possible in vitro to multiply plants that are very difficult to propagate by cuttings or other traditional methods
- Large numbers of genetically identical clones may be produced
- Seeds can be germinated with no risk of damping off/predation
- Under certain conditions, plant material can be stored in vitro for considerable periods of time with little or no maintenance
- Tissue culture techniques are used for virus eradication, genetic manipulation, somatic hybridization and other procedures that benefit propagation, plant improvement, and basic research.
- Tissue culture is an essential part of many genetic transformation protocols.

Plant Tissue Culture Techniques and Applications in Plant Improvement	
Technique	**Applications**
Seed culture	Increasing efficiency of germination of seeds that are difficult to germinate in vivo Precocious germination by application of plant growth regulators Production of clean seedlings for explants or meristem culture
Embryo culture	Overcoming embryo abortion due to incompatibility barriers Overcoming seed dormancy and self-sterility of seeds Embryo rescue in distant (interspecific or intergeneric) hybridization where endosperm development is poor Shortening of breeding cycle

Ovary or ovule culture	Production of haploid plants A common explant for the initiation of somatic embryogenic cultures Overcoming abortion of embryos of wide hybrids at very early stages of development due to incompatibility barriers *In vitro* fertilization for the production of distant hybrids avoiding style and stigmatic incompatibility that inhibits pollen germination and pollen tube growth
Anther and microspore culture	Production of haploid plants Production of homozygous diploid lines through chromosome doubling, thus reducing the time required to produce inbred lines Uncovering mutations or recessive phenotypes
In vitro pollination	Production of hybrids difficult to produce by embryo rescue
Organ culture	Any plant organ can serve as an explant to initiate cultures
Shoot apical meristem culture	Production of virus free germplasm Mass production of desirable genotypes Facilitation of exchange between locations (production of clean material) Cryopreservation (cold storage) or *in vitro* conservation of germplasm

Somatic embryogenesis	One major path of regeneration Mass multiplication Production of artificial seeds As source material for embryogenic protoplasts Amenable to mechanization and for bioreactors
Organogenesis	One major path of regeneration Mass multiplication Conservation of germplasm at either normal or sub-zero temperatures
Enhanced axillary budding	Micropropagation
Callus Cultures	In some instances it is necessary to go through a callus phase prior to regeneration via somatic embryogenesis or organogenesis For generation of useful somaclonal variants (genetic or epigenetic) As a source of protoplasts and suspension cultures For production of metabolites Used in i*n vitro* selection
In vitro mutagenesis	Induction of polyploidy Introduction of genetic variability
Protoplast isolation, culture and fusion	Combining genomes to produce somatic hybrids, asymmetric hybrids or cybrids Production of organelle recombinants Transfer of cytoplasmic male sterility

In vitro flowering	This can be done in some instances but I am not sure there are any practical applications
Micrografting	Overcoming graft incompatibility Rapid mass propagation of elite scions by grafting onto rootstocks that have desirable traits like resistance to soil-borne pathogens and diseases To allow survival of difficult to root shoots Development of virus free plants
Genetic transformation	Many different explants can be used, depending on the plant species and its favored method of regeneration as well as the method of transformation Introduction of foreign DNA to generate novel (and typically desirable) genetic combinations Used to study the function of genes