Plant tissue culture

Plant tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (in vitro). Tissue culture produces clones, in which all product cells have the same genotype (unless affected by mutation during culture).

- ❖ 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.
- ❖ The first commercial use of plant tissue culture on artificial media was in the germination and growth of orchid plants, in the 1920's.
- ❖ In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (Murashige & Skoog, 1962) that plant tissue culture really 'took off' commercially.
- ❖ Tissue culture techniques are used for **virus eradication**, **genetic manipulation**, **somatic hybridization** and other procedures that benefit propagation, plant improvement and basic research.

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.
- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.

- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and *Nepenthes*.
- To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

  - 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.

Tissue culture has several critical requirements:

• Appropriate tissue (some tissues culture better than others)

• A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid

• Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can overrun a culture.

• Growth regulators - in plants, both auxins & cytokinins.

• Frequent subculturing to ensure adequate nutrition and to avoid the build-up of waste metabolites.

Appropriate tissue (Explant)

- Explants: Cell, tissue or organ of a plant that is used to start in vitro cultures. Many different explants can be used for tissue culture, but axillary buds and meristems are most commonly used.

- The explants must be sterilized to remove microbial contaminants. This is usually done by chemical surface sterilization of the explants 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.

Nutrition medium

When an explant 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 nutrient medium is defined by its mineral salt composition, carbon source, vitamins, plant growth regulators and other organic supplements.
- pH determines many important aspects of the structure and activity of biological macromolecules. Optimum pH of 5.0-6.0 tends to fall during autoclaving and growth

Mineral salt composition

- Macroelements: The elements required in concentration > 0.5 mmol/l
  The essential macroelements: N, K, P, Ca, S, Mg, Cl
- Microelements: The elements required in conc. < 0.5 mmol/l
  The essential microelements: Fe, Mn, B, Cu, Zn, I, Mo, Co

The optimum concentration → maximum growth rate

Carbon sources and vitamins

- ✓ Sucrose or glucose (sometimes fructose), concentration 2-5%
- ✓ Most media contain myo-inositol, which improves cell growth
- ✓ An absolute requirement for vitamin B1 (thiamine)
- ✓ Growth is also improved by the addition of nicotinic acid and vitamin B6 (pyridoxine)
- ✓ Some media contain pantothenic acid, biotin, folic acid, p-amino benzoic acid, choline chloride, riboflavine and ascorbic acid (C-vitamin)

Plant growth regulators (Body building Plants)

Auxins:

- induces cell division, cell elongation, swelling of tissues, formation of callus, formation of adventitious roots.
- inhibits adventitious and axillary shoot formation - 2,4-D, NAA, IAA, IBA, pCPA…

Cytokinins:

- shoot induction, cell division
- BAP, Kinetin, zeatin, 2iP…

Gibberellins:

- plant regeneration, elongation of internodes
- GA3…

Abscisic acid:

- induction of embryogenesis
- ABA

Organic supplements

- **N** in the form of amino acids (glutamine, asparagine) and nucleotides (adenine)
- **Organic acids:** TCA cycle acids (citrate, malate, succinate, fumarate), pyruvate
- **Complex substances**: yeast extract, malt extract, coconut milk, protein hydrolysate
- **Activated charcoal** is used where phenol-like compounds are a problem, absorbing toxic pigments and stabilizing pH. Also, to prevent oxidation of phenols PVP (polyvinylpyrrolidone), citric acid, ascorbic acid, thiourea and L-cysteine are used

Applications

Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.

- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.
- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virus stock, such as sugarcane, potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.
- Large scale production of artificial seeds through somatic embryogenesis.